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THIS ISSUE IS DEDICATED

TO

YRJÖ REENPÄÄ

PROFESSOR OF PHYSIOLOGY AT THE UNIVERSITY OF HELSINKI

ON THE OCCASION

OF HIS SIXTIETH ANNIVERSARY

18TH JULY 1954

From the Institute of Physiology, University of Helsinki.

On the Protracted Action of Histamine on the Oxygen Consumption in Rats.

By

A. N. I. APAJALAHTI and S. PIHA.

Received 15 January 1954.

The effect of histamine on the oxygen consumption of mammals has been studied by relatively few investigators, and the results obtained are somewhat inconsistent. ABELIN (1922) observed that 7—20 mg of histamine/100 g body weight did not influence the oxygen consumption at external temperatures from 18 to 20° C. EPPINGER and his coworkers (1928) using anaesthetized dogs showed that during the first ten minutes period oxygen consumption fell but thereafter it gradually increased markedly over the normal level. KNIPPING and STEIGER (1929) too, working with dogs, observed after subcutaneous injection of 15 to 20 mg of histamine a considerable elevation in basal metabolism.

The effect of histamine administered subcutaneously on the basal metabolism of human subjects has been investigated by some workers. Both decreasing and increasing effects have been reported (GRAB 1928, JAHN 1928, VON EULER and LILJESTRAND 1929, and DZSINICH and PELY 1934). The last mentioned authors explained the decreasing effect of histamine on the basis of stimulation of the parasympathetic and paralysis of the sympathetic nervous systems.

WEISS and coworkers (1929, 1932) and later PETERS and coworkers (1944, 1945) were the first to administer histamine as a continuous intravenous infusion. During infusion of 0.02 to 0.12 mg of histamine per minute WEISS and his coworkers found an

elevation of the calory production and oxygen consumption in human subjects, which returned to the original level after the infusion was discontinued. According to PETERS and coworkers the increase of oxygen consumption was roughly proportional to the rate at which histamine was given (0.006 to 0.048 mg per minute). The authors suppose that both the dilation of the arterioles and capillaries caused by histamine and its action as a secretagogue require the expenditure of energy and play a part in the increased oxygen consumption. FABINYI and SZEBEHELY (1948, 1949) noted, on the other hand, that an injection of histamine (5 mg/100 g) decreased the body temperature of mouse and rat at 20°, but increased the oxygen consumption and body temperature at outer temperatures of 30—33° C. ISSEKUTZ Jr. and coworkers (1950) observed that the body temperature decreasing effect of histamine at 20° is related to the loss of heat, brought about by the dilating of the skin capillaries. GYERMEK (1950) demonstrated that the increasing effect of histamine on oxygen consumption depends on secondary mobilisation of adrenaline. The rôle of external temperature in histamine treated animals is supported by investigations on antihistamines. FABINYI-SZEBEHELY and SZEBEHELY (1952), AMBRUS and coworkers (1951) for instance, have noted that antihistamines (diphenylhydramine and tripelenamine) prevent, in rats and mice, the body temperature and oxygen consumption increasing effect of histamine at 30—33°C, and the body temperature decreasing effect of 20° C. On the other hand, antihistamines themselves cause a decrease in the body temperature (PACKMAN et al. 1953).

These results are based on experiments of very short duration. The experimental period has varied from a few minutes to 5—6 hours, and histamine has usually been administered in one subcutaneous injection or as continuous intravenous infusion of 5—10 min. duration. Therefore it was thought necessary to study the oxygen consumption during prolonged action of histamine. As the method of ISSEKUTZ (1942) requires the use of anaesthetized animals it was not considered suitable for the present experiments. Therefore the method of KROGH and LINDBERG (1931) was adapted for the determination of the oxygen consumption of several rats simultaneously. This procedure reduces according to the studies of LUNDHOLM (1949) the variations, which otherwise are quite considerable. By omitting artificial circulation of air in the system, which tends to disquiet the rats, a further reduction of the variations was effected.

Methods and Material.

The apparatus for determination of oxygen consumption consists of: 1. Desiccator of 12 liters capacity with a thermometer to be read with an accuracy of 1/20° C. The desiccator is connected with a spirometer and, by means of a stopcock, with the outside air. In the desiccator the rats are placed in a common cage, each in a separate box with non-transparent walls. The absorption fluid is placed on the bottom of the desiccator. 2. Spirometer of 400 cc capacity connected with the desiccator and, by means of a 3-way stopcock, to an oxygen bag and to a 50 cc syringe for calibration. 3. Kymograph.

Procedure. The rats are placed into the desiccator and the spirometer is filled with oxygen. The conditions in the desiccator are then allowed to stabilize for 20 minutes (rats, temperature, water vapour etc.). During this period the amount of gas which corresponds to the increase of volume of air and to the developing of water vapour is removed from the desiccator and replaced by oxygen from the spirometer in order to restore the oxygen partial pressure of air. At the end of the adaptation period the spirometer is refilled with a sufficient amount of oxygen from the oxygen bag.

Immediately before the experiments the apparatus is calibrated by means of introducing successive samples of 50 cc into the desiccator and drawing the corresponding lines on the drum of the kymograph.

After adaptation for 20 min. the pen of the spirometer is placed against the drum and the measurement is begun. The points at which the experimental curve cuts the calibration lines are recorded with a stopwatch. The time taken by the consumption of 250 cc of oxygen is measured; as a criterion for the accuracy of the determination the equality of the times for consumption of each 50 cc is considered. The temperature in the desiccator is read at the beginning and at the end of the experiment. In order to determine the effect of temperature changes on the gas volume in apparatus, a hot-water bottle is placed into the desiccator and the experiment is performed as described above.

Several successive determinations were carried out in order to obtain good accuracy, and as the experimental value the average of those experiments in which the ratio volume increase/time was approximately linear was chosen. Four rats were placed in the desiccator at a time. As absorption liquid 500 cc of 5 % sodium hydroxide was used and the liquid was changed each morning or every 3 hours during the experiment.

Powdery histamine dihydrochloride was added into a mixture of bee's wax and mineral oil according to the method of Code and Varco (1942); 1 cc of the mixture contained 100 mg of free histamine base. 400 mg/kg body weight heated to 39° C were injected subcutaneously into the neck of the rats. The injection did not call forth any symptoms of shock or discomfort. The deposite vanished slowly within a week; no signs of general or local irritation were observed.

The experiments were performed on 12 rats kept on carbohydrate diet consisting of dried bread and water. The diet was begun about a

week before the first measurement. The rats weighed 220—280 g; during the experimental period there were no considerable weight changes. Special care was taken to handle the rats quietly and always with bare hands.

Results.

When the oxygen consumption of untreated rats was measured from day to day the values did not vary by more than 3 %. This accuracy was found sufficient for the present experiments. Three

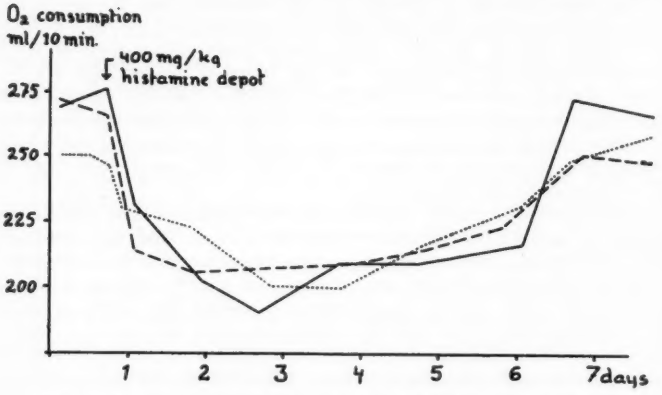

Fig. 1. Oxygen consumption in rats after histamine administration.

series of experiments were performed altogether, each comprising four histamine-treated rats. The first measurements were carried out about half an hour after the administration of histamine, and then after suitable intervals for approximately one week. The rats were kept in a room at a temperature of 22° C during the whole experimental period. The temperature in the desiccator was stabilized at 27—28°C. The results are represented in figure 1 in which the oxygen consumption of four rats is plotted against time. A relatively even decrease in the oxygen consumption beginning about 2—5 hours after histamine administration is observed. A minimum is reached after approximately 50 hours, the values then remaining at a low level for about a week. Thereafter a slow increase is noted until the initial level is restored. The minimum of the oxygen consumption is remarkably low, only about 75 % of the initial value. In one of the experimental series a transient increase of ca. 30

Table 1.

Initial oxygen consumption at 22° C ml/10 min.	Oxygen consumption at 18° C about 20 hours after histamine administration ml/10 min.	Difference %	Oxygen consumption at 22° C 4 hours after restoration of the room temperature ml/10 min.	Difference %	Test animals
245	254	+ 4	245	0	4 control rats
266	278	+ 4.5	266	0	4 control rats
245	258	+ 5.5	217	— 11.5	4 histaminized rats
245	264	+ 8.2	227	— 8	4 histaminized rats

min. duration was noted half an hour after administration of histamine. Because of its brief duration it is not visible on the chart (figure 1).

It was observed that the temperature of the animal room in which the rats were kept affected the oxygen consumption particularly of the histaminized rats. The data are given in table 1. At a room temperature of 18° C the oxygen consumption was not decreased in spite of histamine administration. When the temperature of the animal room was restored to 22° C the oxygen consumption of the rats which had received histamine fall below the initial level, whereas in the controls it only returned to that level.

Discussion.

To summarize the results of the earlier investigations, histamine in certain conditions seems to increase the oxygen consumption. This is, however, interpreted as a result of a secondary mobilisation of adrenaline, while histamine is usually not considered to have any direct effect on the oxygen consumption. In the present experiments a marked decrease in the oxygen consumption was observed if the rats were kept under the prolonged action of histamine at a room temperature of 22° C. The decrease could not possibly have been observed in the earlier experiments, as the histamine administered in a single injection must have been wholly destroyed in the organism because the enzymatic breakdown of histamine is very rapid (EMMELIN 1951).

The fact that in the present study no decrease was noted at a low temperature is only in apparent conflict with the regular

decrease at higher room temperature. As well known, histamine dilates the capillaries of the skin and increases consequently the heat loss at low external temperature. In order to keep the body temperature constant the oxygen consumption must be increased and the decrease in oxygen consumption manifested at higher temperatures after prolonged histamine administration will be compensated. Therefore it is not adequate to work with anaesthetized animals in which the temperature regulation is deficient. The inconsistent results of earlier investigators may be partly explained by this fact.

A secondary mobilisation of adrenaline may explain the transient rise in the oxygen consumption observed in one of our experiments. According to the present results the essential effect of prolonged histaminization appears to be a decrease in oxygen consumption.

Summary.

The oxygen consumption of 12 rats was determined after they had received a subcutaneous injection of histamine in bee's wax. The histamine deposit caused a decrease in the oxygen consumption to an average minimum at 76 % of the normal value when the rats were kept at a room temperature of 22° C. The minimum was reached approximately after 50 hours. This decrease does not appear when rats are kept at a temperature of 18° C.

References.

Abelin, J., Biochem. Z. 1922. *129*. 1.
Ambrus, J., C. Ambrus and J. Jacob, Arch. int. Pharmacodyn. 1951. *86*. 350.
Code, C. F. and R. L. Vargo, Amer. J. Physiol. 1942. *137*. 225.
Dzsinich, A. and M. Pély, Klin. Wschr. 1934. *13*. 699.
Emmelin, N., Acta physiol. Scand. 1951. *22*. 379.
Eppinger, H., D. Laszlo and A. Schürmeyer, Klin. Wschr. 1928. *7*. 2231.
v. Euler, M. and G. Liljestrand, Scand. Arch. Physiol. 1929. *55*. 1.
Fabinyi, M. and J. Szebehely, Arch. int. Pharmacodyn. 1948. *75*. 402.
— — Acta allerg. 1949. *2*. 233.
— — Schweiz. med. Wschr. 1949. *72*. 299.
Fabinyi-Szebehely, M. and J. Szebehely, Acta physiol. Scand. 1952. *27*. 1.

GRAB, W., Z. ges. exper. Med. 1928. *63*. 360.
GYERMEK, L., Arch. exp. Path. Pharmak. 1950. *209*. 456.
ISSEKUTZ, B. sen. and B. ISSEKUTZ jun., Ibidem 1942. *199*. 306.
ISSEKUTZ, B. jr., I. LICHTNECKERT and H. NAGY, Arch. int. Pharmacodyn. 1950. *81*. 35.
JAHN, D., Arch. klin. Med. 1928. *159*. 152.
— Ibidem 1928. *159*. 335.
KNIPPING, H. W. and J. STEIGER, Z. ges. exp. Med. 1929. *64*. 594.
KROGH, M. and A. L. LINDBERG, Bibl. f. Laeger 1931. *123*. 361.
LUNDHOLM, L., Acta physiol. scand. 1949, *18*. 259.
PACKMAN, E. W., G. V. ROSSI and J. W. E. HARRISSON, J. Pharm. Pharmacol. 1953. *5*. 301.
PETERS, G. A. and B. T. HORTON, Proc. Centr. Soc. Clin. Res. 1944. *17*. 65.
— B. T. HORTON and W. M. BOOTHBY, J. Clin. Invest. 1945. *24*. 611.
WEISS, S., L. B. ELLIS and G. ROBB, Amer. J. Physiol. 1929. *90*. 551.
— G. B. ROBB and L. B. ELLIS, Arch. int. Med. 1932. *49*. 360.

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Monosynaptic Corticospinal Activation of Fore Limb Motoneurones in Monkeys (Macaca mulatta).

By

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Received 20 January 1954.

In earlier publications (BERNHARD, BOHM and PETERSÉN 1953 a and b) experiments on monkeys (*Macaca mulatta*) were described, in which the precentral cortical area was stimulated and the action potentials were led off from the lumbar ventral roots. When a train of square wave shocks of short duration and a frequency of *e. g.* 20 per sec. was used, it was found that 2—3 seconds after the beginning of the repetitive stimulation, each stimulus was followed by a series of action potentials in the lumbar roots contralateral to the cortical area stimulated. As the latency of the earliest ventral root response following each shock was remarkably short, we thought that it might indicate a monosynaptic transmission between the corticospinal fibres and the spinal motoneurones. In order to estimate the time for conduction from the cortex to the lumbar segments in the pyramidal fibres with highest conduction velocity (the corticospinal conduction time) the latency of the response to cortical stimulation in the lumbar part of the pyramidal tract was measured as well as the conduction time in the motoneurones from the spinal cord to the recording electrode on the ventral root. When the corticospinal conduction time and the time for conduction in the ventral root were subtracted from the latency of the earliest ventral root response to cortical stimulation, an average of 0.7 msec. was obtained. Therefore it was concluded that spinal motoneurones

in the lumbar region, contralateral to the cortical area stimulated, can be monosynaptically activated by descending volleys in the corticospinal fibres with the highest conduction velocity. A comparison was made between the characteristic behaviour during the repetitive stimulation of the early motoneurone response in different nerves of the upper and lower extremities. These experiments indicated that the motoneurones innervating fore limb muscles can also be monosynaptically activated by descending volleys in the corticospinal fibres.

The present investigations were carried out in order to obtain further evidence of the monosynaptic activation of the fore limb motoneurones from the corticospinal neurones.

Methods.

The experiments were carried out under light dial or nembutal anaesthesia and small doses of d-tubocurarine were administered. The skull and spine were fixed by means of clamps and the extremities with bone drills. The skull was opened and a paraffin oil pool (38° C) was arranged over the cortex, the dura being removed under the paraffin oil. After laminectomy the spinal cord was covered with paraffin oil, as were the peripheral nerves from which the action potentials were led off. Electrical square wave shocks (duration 0.2—1 msec.) were used for stimulation.

Results.

As mentioned above repetitive cortical stimulation with a frequency of 15—20 per sec. was found to be convenient for the elicitation of the short latency motoneurone response to each individual cortical stimulus. Fig. 1 shows the typical action potentials in *N. radialis*, when the contralateral fore limb subdivision of the precentral motor area was stimulated with a train of shocks (0.8 msec. duration) at a frequency of 20 per sec. Each picture (A—F) in Fig. 1 shows a series of superimposed records of the action potentials following upon each stimulus at different intervals after the beginning of the repetitive stimulation. In Fig. 1 A taken at the beginning of the stimulation period there was only a late response, the latency of which was about 5.5

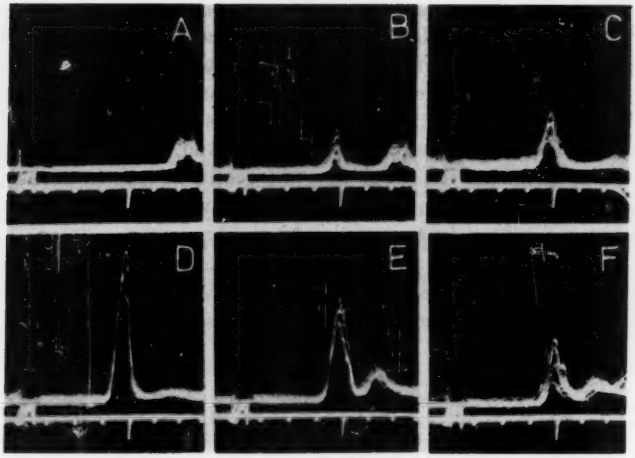

Fig. 1. Superimposed action potentials in *N. radialis* following each stimulus in a train of shocks (stimulus frequency 20 per sec.) delivered to the fore limb subdivision of the contralateral motor area. A at the beginning of stimulation; B—F 2, 3, 4, 7 and 8 sec. after beginning of stimulation. Time in msec.

msec. Two seconds after the onset of the repetitive stimulation an early response appeared (see Fig. 1 B), which increased (Fig. 1 C) and reached a maximal amplitude after about four sec. (Fig. 1 D) following the onset of the repetitive cortical stimulation. Later on the amplitude of the early response decreased (Fig. 1 E—F). The successive increase and subsequent decrease of the amplitude of the early response during the stimulation period is typical (*cf.* BERNHARD, BOHM and PETERSÉN, 1953 b). The effect of different stimulation frequencies on the development of the early response, its distribution to different nerves as well as its behaviour when different cortical points are stimulated will be shown in a following paper (BERNHARD and BOHM). In this connection interest will be focused on the latency of the early response only, which, as seen in Fig. 1 D, was about 3.2 msec.

The records in Fig. 2 are from the same experiment, and Fig. 2 A again shows a series of records of the action potentials in *N. radialis* obtained when the amplitude of the short latency response to contralateral cortical stimulation had reached its maximum. The average value of the latency for the early response

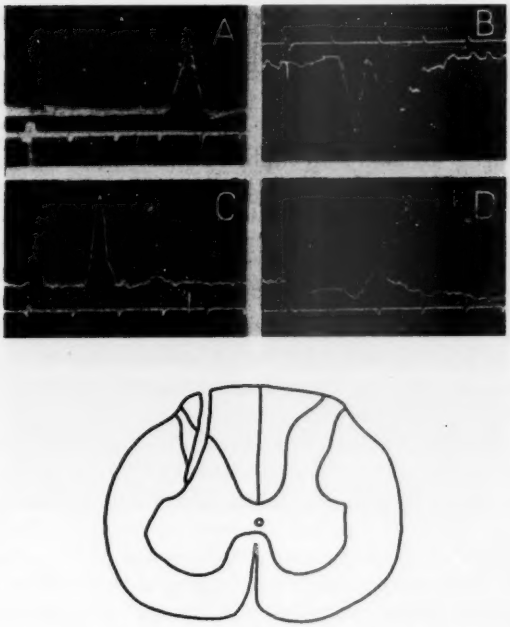

Fig. 2 A, action potentials in *N. radialis*, B in the lateral pyramidal tract at the C_7 segmental level (see diagram below) following contralateral cortical stimulation. C response in *N. radialis* following motoneurone stimulation at the C_7 segmental level. D cortical response in the fore leg subdivision to antidromic stimulation of the pyramidal tract at the C_7 segmental level. Time in msec.

was found to be 3.2 msec. The action potentials were then led off with a needle electrode from the pyramidal tract at the C_7 segmental level (see needle track in cervical cord section in Fig. 2) ipsilateral to the nerve, from which the records in Fig. 2 A were taken. Fig. 2 B shows the tract response to cortical single shock stimulation, the stimulating electrode being placed on the same cortical point as in the experiment illustrated in Fig. 2 A. The latency of the first response was on an average 1.2 msec. In order to estimate the conduction time in the motoneurones, which were activated indirectly by cortical stimulation in Fig. 2 A, the dorsal roots were cut and the action potential was recorded from *N. radialis* (same electrode position as in 2 A), when the motoneurones were stimulated directly by the needle elec-

trode being inserted into the spinal cord at the C_7 segmental level. Fig. 2 C shows that the latency of the nerve response was 1.3 msec., which value represents the conduction time in the fast conducting motoneurones of *N. radialis*. The subtraction of the sum (2.5 msec.) of the corticospinal conduction time (1.2 msec.; Fig. 2 B) and the peripheral conduction time (1.3 msec.; Fig. 2 C) from the latency of the early response in *N. radialis* to contralateral cortical stimulation (3.2 msec. in Fig. 2 A) thus gave a value of 0.7 msec.

Serial sections of the spinal cord at the C_7 segmental level showed that in the experiment illustrated in Fig. 2 B the tip of the recording electrode had been placed in the pyramidal tract. Therefore it was concluded that the latency of the tract response to cortical stimulation represented the conduction time in corticospinal fibres with the highest conduction velocity. It may be argued that this response represents activity in other fibres that in those belonging to the direct corticospinal tract *e. g.* in descending fibres from subcortical regions, which may be indirectly activated from the cortical neurones stimulated. Owing to a high conduction velocity in such bulbo-spinal fibres a descending volley in them might reach the segmental level before a direct corticospinal volley, although the former is transmitted via synapses between the cortical neurones stimulated and the neurones from which the response is obtained at the segmental level.

It was, however, shown that a cortical response to an antidromic stimulation of the spinal tract fibres could easily be obtained with the needle electrode that had previously been used as the recording electrode. Thus, the cortical response within the fore limb subdivision to the antidromic stimulation was led off with the cortical electrode that had previously been used as the stimulating electrode, the needle electrode in the spinal cord being used for stimulation. The positions of the electrodes were not changed. The antidromic response shown in Fig. 2 D is of the same general shape as that obtained by antidromic stimulation of the pyramids by WOOLSEY and CHANG (1947). The latency of the cortical response (Fig. 1 D) to the antidromic stimulation at the C_7 segmental level was found to be the same as in Fig. 2 B.

The cortical response to the antidromic stimulation at the cervical segments has a widespread precentral distribution as

the fibres running to the whole hind part of the body ipsilateral to the stimulating electrode are also activated. In order to demonstrate the specificity of the antidromic response the stimulating needle was inserted into the pyramidal tract at the L_1 level. At this level the pyramidal tract only comprises fibres to the hind leg and tail. Fig. 3 A and B show the action potentials recorded with the needle in the pyramidal tract at the L_1 level

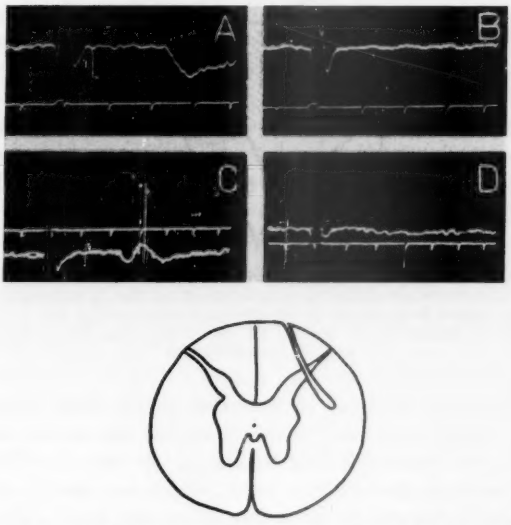

Fig. 3. Records A and B obtained with a needle electrode in the lateral pyramidal tract at the L_1 segmental level (see diagram) when the contralateral upper (A) and lower (B) parts of the precentral area were stimulated. Records C and D obtained from the hind limb (C) and fore limb (D) subdivisions of the precentral motor area when the contralateral pyramidal tract at the L_1 segmental level was stimulated. Time in msec.

(needle track shown in Fig. 3) to cortical stimulation of the upper (3 A) and lower (3 B) parts of the precentral motor area. As seen there is a response only in Fig. 3 A. The stimulating electrode was then used for recording and *vice versa*. Records 3 C and D show the antidromic responses to spinal stimulation from the corresponding cortical points. The response to antidromic stimulation was pronounced when recorded from the upper precentral region (Fig. 3 C) whereas no response was obtained from the lower part of this region (Fig. 3 D). The latency

of the spinal response to orthodromic stimulation in Fig. 3 A was the same as that of the cortical response to antidromic stimulation in Fig. 3 C, *i. e.* 2.5 msec. The distribution of the antidromic response as well as the cortical region from which the pyramidal spinal cord response could be evoked was mapped and the results are illustrated in Fig. 4. The field from which the pyramidal spinal responses at the L_1 segmental level could

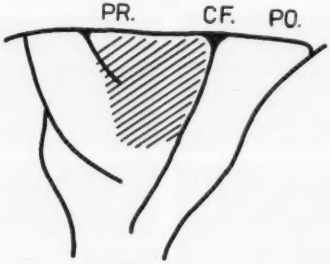

Fig. 4. Shadowed field shows the distribution of the antidromic response to stimulation of the contralateral pyramidal tract at the L_1 segmental level as well as the region from which pyramidal tract responses at the L_1 segmental level could be elicited in the cortex. PR precentral area, PO postcentral area and CF central fissure.

be obtained was found to be the same as that from which early cortical responses to antidromic pyramidal stimulation could be recorded (the shadowed field in Fig. 4). As seen the field comprises the upper and middle parts, which are described as the hind leg and tail subdivisions (see WOOLSEY *et al.* 1950).

Discussion.

When the fore limb subdivision of the precentral motor area was stimulated by means of single shocks of short duration, a series of action potentials were recorded with a needle electrode inserted into the lateral part of the spinal cord on the contralateral side at the C_7 segmental level. The initial part of this response consisted of rapid positive deflections (*cf.* LLOYD's experiments on cats 1941). The fact that the latency of the cortical response to antidromic stimulation (Fig. 2 D) was the same as that of the orthodromic response (Fig. 2 B) strongly favours the view that the latency of the response to cortical stimulation recorded with the needle electrode in the pyramidal tract repre-

sents the conduction time in the corticospinal fibres with the highest conduction velocity. Furthermore the mapping experiment illustrated in Figs. 3 and 4 shows that the neurones concerned are corticospinal fibres. When taking into account the conduction distance in the different experiments, a conduction velocity of about 70 M per sec. was obtained for the most rapidly conducting fibres (*cf.* BERNHARD, BOHM and PETERSÉN, 1953 b). This value is in good correspondence to the diameter values of the largest pyramidal fibres in *Macaca mulatta* given by HÄGGQVIST (1937 a) and LASSEK (1943). According to histological investigations by BRODAL and WALBERG (1952) and electrophysiological studies by BRODAL and KAADA (1953) ascending fibres are found to be present in the pyramid of the cat. This may also be the case as regards monkeys. The value for the conduction velocity obtained in our experiment shows that the latencies measured in our investigations do not concern ascending fibres in the pyramidal tract, the highest conduction rate of which is 50 M per sec. according to BRODAL and KAADA (1953).

The difference between the total latency of the earliest motoneurone response in *N. radialis* and the sum of the values for the corticospinal and peripheral conduction time was found to vary between 0.6 and 0.8 msec. in the different experiments. This value, representing the synaptic delay between the corticospinal neurones and the motoneurones, is of the same magnitude as that found for the synaptic activation of the oculomotor neurones (LORENTE DE NÓ, 1935) and the monosynaptic reflex activation of the spinal motoneurones (ECCLES and PRITCHARD, 1937, RENSHAW 1940, LLOYD 1943). In this connection it should be mentioned that we have recorded the nerve response to contralateral cortical stimulation from two separate points on the nerve (*N. medianus*) in order to control that it was mediated in the motoneurones with the highest conduction velocity. We found that the early motoneurone discharge to contralateral cortical stimulation was conducted with a velocity of 80 M per sec. This value is in good correspondence to the diameter values of the largest ventral root fibres in *Macaca mulatta* given by HÄGGQVIST (1937 b).

Thus, evidence shows that the spinal motoneurones innervating fore limb muscles can be monosynaptically activated by descending volleys in corticospinal fibres originating in the fore limb subdivision of the contralateral motor cortex. The degener-

ation experiments by HOFF and HOFF (1934) offer a morphological background to our observations.

Some of our earlier experiments indicate that there is also an ipsilateral monosynaptic activation of spinal motoneurones from corticospinal fibres. Later experiments confirm this conclusion and these will be published in connection with a detailed investigation of the cortical representation of this "cortico-motoneuronal system" for different muscles (BERNHARD and BOHM).

Summary.

Various types of experiments were carried out on monkeys (*Macaca mulatta*), the results of which show that spinal motoneurones innervating fore limb muscles are monosynaptically activated by descending volleys in corticospinal fibres belonging to the largest pyramidal neurones originating in the contralateral precentral area.

References.

BERNHARD, C. G. and E. BOHM, Arch. Neurol. Psychiat. In press,
BERNHARD, C. G., E. BOHM and I. PETERSÉN, Experientia 1953 a. *9*. 111.
BERNHARD, C. G., E. BOHM and I. PETERSÉN, Acta Physiol. Scand. 1953 b. *29*. Suppl. 106. Page 79.
BRODAL, A. and B. R. KAADA, J. Neurophysiol. 1953. *16*. 567.
BRODAL, A. and F. WALBERG, Arch. Neurol. Psychiat. 1952. *68*. 755.
ECCLES, J. C. and J. J. PRITCHARD, J. Physiol. 1937. *89*. 43.
HÄGGQVIST, G., Acta Psychiat. Kbh. 1937 a. *12*. 457.
— Z. mikr.-anat. Forsch. 1937 b. *42*. 33.
HOFF, E. C. and H. E. HOFF, Brain 1934. *57*. 454.
LASSEK, A. M., J. Comp. Neurol. 1934. *79*. 407.
LLOYD, D. P. C., J. Neurophysiol. 1941. *4*. 525.
— Ibidem 1943. *6*. 110.
LORENTE DE NÓ, R., Amer. J. Physiol. 1935. *111*. 272.
RENSHAW, B., J. Neurophysiol. 1940. *3*. 373.
WOOLSEY, C. N. and H. T. CHANG, Res. Publ. Ass. Nerv. Ment. Dis. 1947. *27*. 146.
WOOLSEY, C. N., P. H. SETTLAGE, D. R. MEYER, W. SENCER, T. P. HAMUG and M. TRAVIS, Res. Publ. Ass. Nerv. Ment. Dis. 1952. *30*. 238.

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Catechol Amines and Substance P in the Mammalian Eye.

By

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Determination of the catechol amine content of the various parts of the eye would seem to offer information as to the extent and distribution of the sympathetic nerve supply to these structures. The central nervous system, of which the retina and the optic nerve can be considered a part, contains only small amounts of catechol amines (HOLTZ 1949) with the exception of certain parts (VOGT 1953). The ciliary body and the iris with an abundant adrenergic nerve supply would be expected to contain considerable amounts of noradrenaline (EULER 1951). Sympathomimetic activity has previously been demonstrated in the aqueous humour (BACQ 1933, LUCO and LISSÁK 1938). We have also studied the occurrence of substance P in the retina. Relatively high amounts of this substance are present in the grey matter of the central nervous system (EULER and GADDUM 1931, PERNOW 1953).

Material and methods.

The material consisted of cow, horse, and dog eyes removed immediately after the killing of the animals and brought to the laboratory within an hour. No difference in results was noted whether the eyes were kept on ice or at ambient temperatures before preparation.

The various parts of the eyes were dissected out and the catechol amines extracted with about 10 volumes of 5 per cent trichloracetic acid, followed by adsorption on aluminum hydroxide. The extracts

were then assayed for adrenaline and noradrenaline on the cat's blood pressure and the fowl's rectal caecum (EULER 1949).

Substance P was extracted by boiling the retina in 2—10 volumes of water, kept at pH 4 with sulfuric acid. Extracts from cow retinae were tested immediately after filtering and neutralization with NaOH. Extracts from dog retinae were first concentrated to dryness *in vacuo* at room temperature, and dissolved in distilled water before testing. The extracts were tested on the isolated guinea-pig's ileum with the addition of atropine (1 in 2.5 millions) and an antihistamine (Lergigan, 1 in 2.5 millions) to the bath fluid. (PERNOW 1951, FELDBERG and coworkers 1952.)

The estimations were compared with a standard preparation obtained from cow's intestine by salting out with 70 per cent saturated ammonium sulphate (EULER 1936).

The specificity of the substance P activity was shown by a) precipitation in some cases with ammonium sulphate giving a yield of 50—60 %, and by b) inactivation with chymotrypsin. To 50 units of substance P from dog retina in ml Tyrode's solution (pH 8.3) was added 0.05 mg crystalline chymotrypsin and the mixture incubated for $^1/_2$ hour at 38 ° C. Total inhibition of the activity was obtained in each case. The activity was expressed in units (EULER 1942), one unit corresponding to the effect of 7—10 threshold doses on isolated guinea-pig ileum in 3 cc Tyrode's solution according to the present assay technique.

The wet weight of the retina of the cow was about 0.5 g. The dry weight of the retina, determined in two experiments, was 15 and 13 per cent of the wet weight. Usually about 20 retinas were used for each preparation.

Results.

I. Catechol amine estimations.

a) Retina.

20 retinae from cow were extracted, prepared as above and assayed. The noradrenaline content amounted to less than 0.05 μg per g tissue and the adrenaline content less than 0.005 μg per g.

b) Aqueous humour.

Aqueous humour was withdrawn from the anterior chamber with a syringe needle and prepared as above. When the extracts were tested on the cat's blood pressure a depressor effect was noted in some instances but never a rise in blood pressure, even with doses corresponding to 20 ml aqueous humour (effect less

than that of 0.05 μg noradrenaline). A slight action corresponding to 1—2 mμg adrenaline per ml was observed on the fowl's rectal caecum. Hydrolyses at pH 1.5 for 20 minutes at 100° C prior to adsorption on aluminum hydroxide did not increase the activity.

In one extract of pooled aqueous humour from cow and horse eyes the noradrenaline was 13 mμg per ml and the adrenaline 7.5 mμg per ml. While it was not possible to demonstrate regularly the presence of catechol amines biologically in absorbed extracts, untreated aqueous humour caused effects on the isolated rabbit's jejunum and on the fowl's rectal caecum corresponding to about 0.05—0.2 μg l-noradrenaline per ml.

c) Lens and vitreous body.

In no instance was it possible to detect any noradrenaline or adrenaline in the extracts tested. The content of total catechol amines would therefore be less than 0.05 μg noradrenaline equivalents per 100 g.

d) Ciliary body, iris and choroid.

The ciliary body and iris were prepared together and separated from the choroid.

Two preparations of each were made, each comprising the organs from 20 eyes. The results are given in Table I.

Table I.

Catechol amine content in cattle and horse eyes.

Preparation	Noradrenaline	Adrenaline	Per cent adrenaline
Retina	< 0.05 μg/g	< 0.005 μg/g	
Aqueous humour	0 — 1.3 μg/100 ml	0 — 0.75 μg/100 ml	— — —
Lens and Vitreous body	< 0.05 μg/100 g	— —	
Ciliary body and iris.	0.39 μg/g	0.010 μg/g	2.6 %
	0.39 »	0.030 »	7.3 %
Choroid	0.29 »	0.012 »	4.0 %
	0.32 »	0.019 »	5.5 %

II. Substance P in the retina.

The results are tabulated below (Table II).

Table II.

Substance P in retina of cow and dog.

Material	Mode of preparation	Units per retina	Units per g retina	
			wet weight	dry weight
Cow	Boiling water at pH 4, not concentrated	7	13	93
»	as above, but concentrated by boiling	5	10	71
»	as above	5	10	71
»	extracted with 75 per cent ethanol, evaporation to dryness	2.5	5	36
Dog	boiling water pH 4		23	153
»	» »		28	200
»	» »		25	167
»	» »		20	130
»	» »		25	170

Comment.

Of the different parts of the eye only the ciliary body, the iris and the choroid contained noradrenaline in appreciable amounts. The content corresponded roughly to that found in other smooth muscle organs such as blood vessels, uterus and intestine. In agreement with the results obtained from most other mammalian organs the adrenaline percentage was low, 2.6—7.2 per cent of the total catechols.

The low catechol amine figures in the retina speaks against an adrenergic nerve supply to the organ.

It was previously noted (Euler and Porat 1947) that a concentrate of aqueous humour from cow acquired a distinct red colour on alkalization with sodium hydroxide. Such a colour was also seen in one sample of aqueous humour in the present series kept at room temperature in the laboratory for several hours. In this sample the reaction had changed to about pH 9. The

colour suggests the formation of a chrome compound derived from a catechol substance with a weak biological action.

The presence of noradrenaline in aqueous humour might be the result of filtration and/or secretion from the blood plasma or by leakage from the adrenergic nerve endings of the ciliary body and the iris. A difference in the participation of these factors may account for the variations in the results. It seems improbable that the amounts of noradrenaline found came from the blood plasma, since the concentration in arterial blood probably does not far exceed 0.1 μg per 100 ml normally (Lund 1951). A secretion from the adrenergic nerve endings would be, however, in conformity with the results obtained by Bacq (1933) and by Luco and Lissák (1938) under normal conditions and after sympathetic nerve stimulation.

The content of substance P in the retina is high, about 30—90 units per g dry weight in the cow and still higher in the dog, suggesting that the substance may be of functional significance in this organ. These figures are comparable to those obtained in the small intestine of the cow which are on an average 20 units per g wet weight or 80 units per g dry weight (Pernow 1951). Kopera and Lazarini (1953) in a recent study of the distribution of substance P in the central nervous system found figures similar to those obtained by Pernow (1953) but found no substance P activity in cow's retina. Since the extraction procedure was the same as that used by us, the difference in results is difficult to explain.

Summary.

The noradrenaline content of the ciliary body, the iris, and the choroid in the cow was 0.3—0.4 μg per g wet weight. Adrenaline was present to 2.6—7.2 per cent of the total catechols.

In aqueous humour the noradrenaline was generally less than 0.2 μg per 100 ml. On one occasion an effect corresponding to 1.3 μg noradrenaline per 100 ml was found.

Detectable amounts of catechol amines were not found in the lens or the vitreous body.

The retina did not contain measurable quantities of noradrenaline or adrenaline (< 0.05 μg/g).

Concentrated aqueous humour acquires a red colour on standing at alkaline reaction.

The retina of cow and dog contains high amounts of substance P, up to 200 units per gram of dry organ.

References.

Bacq, Z. M., Arch. int. physiol. 1933. *36*. 167.
Euler, U. S. v., Skand. Arch. Physiol. 1936. *73*. 142.
— Acta Physiol. Scand. 1942. *4*. 373.
— Ibidem 1949. *19*. 207.
— Pharmacol. Rev. 1951. *3*. 247.
— and J. H. Gaddum, J. Physiol. 1931. *72*. 74.
— and B. v. Porat, 1947 (unpubl. results).
Feldberg, D. W., W. D. M. Paton, and M. Schachter, J. Physiol. 1951. *115*. 163.
Holtz, P., and H.-J. Schümann, Schweiz. Med. Wschr. 1949. *79*. 252.
Kopera, H., and W. Lazarini, Arch. exp. Path. Pharmak. 1953. *219*. 214.
Lund, A., Thesis, Einar Munksgaard, Köpenhamn 1951.
Luco, J. V., and K. Lissák, Amer. J. Physiol. 1938. *124*. 271.
Pernow, B., Acta Physiol. Scand. 1951. *24*. 97.
— Ibidem 1953. *29*. Suppl. 105.
Vogt, M., Abstr. Commun. XIX Int. Physiol. Congr. Montreal 1953.

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A Method for Enzymic Determination of ATP in Tissues.

By

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We have earlier published a work on determination of ATP in blood employing the enzymic micro-method (REHELL, FORSANDER and RÄIHÄ 1952). As we continued our experiments it was proved necessary also to find a method for determination of adenosine triphosphate in samples from tissues. The determination of samples from tissues is in principle carried out in the same way as the determination from blood samples. With a specific enzyme (LEE and EILER 1951) the end-phosphate group is split from ATP and measured photometrically. While in a determination from blood the quantity of the sample is easily estimated, the size of a tissue sample is not quite so easily verified. In order to solve this problem we tried the following method: We carried out a micro-nitrogen determination from the protein we obtained from the perchloric acid precipitate, and with regard to this value we calculated the phosphorus values.

Method.

Solutions for phosphorus determination:

1) 2 per cent $HClO_4$ • 2.75 ml conc. $HClO_4$ ad 100 ml aq. redest. (Merck pro anal. 70 per cent, sp.g. 1.67).

2) 0.1 M veronal solution. 10.3 g Na-dietylbarbiturate (Bayer) ad 500 ml with CO_2-free water.

3) Enzyme solution (LEE and EILER 1951).

4) 7.5 per cent Na-molybdate aa 10-n H_2SO_4.

5) Stannochloride. 11.9 g $SnCl_2 \cdot 2H_2O$/25 ml conc. HCl pro anal. Diluted before use 1 : 200 with aq. redest.

Nitrogen Determination.

The micro-Kjeldahl nitrogen determination method is used.

Collection and Treatment of Samples.

From the organ in question a small sample of about 100 mg is taken and put immediately into 2 ml of a 2 per cent perchloric acid solution and homogenized. We have used the homogenizer as shown in Fig. 1, which we have made from Pyrex-glass in accordance with our own drawings. In this homogenizer even the toughest samples can be ground to suspension within about 20 seconds. The conical shape of the central part of the homogenizer is combined with a vertical electric motor with a stiff rubber tube. The homogenizer has worked most satisfactorily.

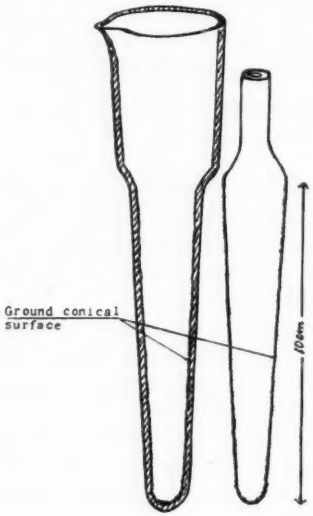

Fig. 1.

After the grinding the suspense is poured from the homogenizer into a small centrifuge tube (10 ml). The homogenizer is rinsed with 2 ml of perchloric acid, the fluid is added to the suspense in the centrifuge tube and centrifuged down for 10 min. at 3,000 r.p.m. The clear solution is poured through a small funnel into a 50 ml bottle. The precipitate in the centrifuge tube is washed with 4 ml of perchloric acid and centrifuged down as before. The clear fluid is added to the first solution. In serial work it is advantageous to do the washing so that the mixing is done with the glass stick fastened to the vertical electric motor. The washing is thus done quickly and effectively without further trouble.

The protein in the centrifuge tube is taken care of for later nitrogen determination.

Thirty-five ml of veronal solution is added to the centrifuged solution and the bottle is filled with aq. redest. to the mark. The in-

organic phosphate which the enzyme splits from ATP is determined from this solution. Exactly 5 ml of the solution is poured into two graduated test tubes, the second one is for the blank. We have used non-protein nitrogen tubes with marked 8.75 and 12.5 ml. The tubes are placed in an 18°C water thermostat, and to the test but not to the blank 0.1 ml of enzyme solution is added (LEE and EILER 1951). The fluid is incubated for exactly 15 min., and after this 2 ml of Na-molybden solution is added plus 1 ml of diluted stannochlorid solution, the latter so that the point of the pipette is dipped deep in the fluid as it flows down into the reagens. The colour is developed in 15 min. and is measured photometrically at a wave length of 740 mm. The values are read from a phosphorus standard curve.

Nitrogen Determination.

For the nitrogen determination the micro-Kjeldahl method is employed. The protein on the bottom of the centrifuge is suspensed with aq. redest. in a 25 ml graduated bottle. For example exactly 2 ml of the suspense is taken and digested. The nitrogen value is converted to protein value by multiplying by 6.25.

Discussion of the Method.

How fast does the ATP split in the isolated tissue sample? This question is of utmost importance as it has to be fixed how soon after the operation the isolated tissue sample has to be brought to the laboratory (Fig. 2).

In these experiments we have used samples from rat heart muscles. The experiments were carried out as follows:

From an isolated, beating heart of a rat a sample was cut out and immediately put into perchloric acid. The remains of the heart muscle were placed on a watch-glass in room temperature, and small samples were cut out at known intervals.

Figure 2 shows that the ATP value, sometimes, can be preserved unchanged for about 15 min., but sometimes it is split in the tissue sample much sooner. Most of the values obtained from rat heart muscle have been of the order 60—90 γ P/100 mg protein but, as is shown in Fig. 2, we have obtained much higher values in some cases.

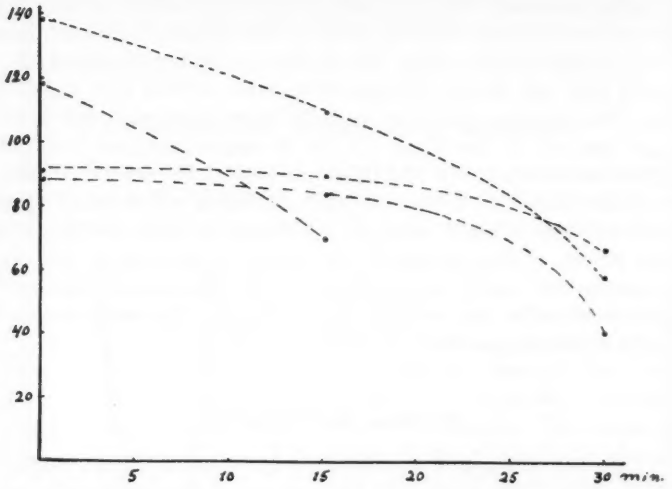

Fig. 2. The splitting of ATP (γ ATP-P/100 mg prot.) in rat heart muscle at room temperature.

The Accuracy of the Method.

In our method for determination of ATP in blood we have presented the limits of error. When the ATP determination is made from the tissues there is, moreover, the possibility of the sample not being quite homogeneous. We have performed parallel determinations from samples from rat heart and abdominal muscles and obtained the results shown in Table 1. In our experiments samples from rat heart and abdominal muscles are isolated and at once put in liquid air. From these refrigerated samples smaller samples of suitable sizes have been cut out, and from these samples the ATP determination has been carried out as described above.

Besides from samples from laboratory animals the ATP content has been determined from samples obtained in the operating theatre. In this case the surgeon has cut out a sample of about 0.5 g which at once has been put into liquid air. From this refrigerated piece of tissue samples of suitable sizes have been cut out and treated as described above. The results are shown in Table 2.

Table 1.

Parallel ATP determinations from rat heart and abdominal muscles. The values are given as γ ATP-P/100 mg protein.

Organ	Parallel experiments			
	1	2	3	4
1. Heart muscle	66	68	51	74
2. »	70	68	70	—
3. Abdominal muscle	140	150	125	147
4. »	183	148	—	—
5. »	171	75	107	—
6. »	107	112	—	—
7. »	147	98	118	—
8. »	75	109	—	—

Table 2.

ATP determinations from samples obtained in the Operating Theatre. The values are given as γ ATP-P/100 mg protein.

Age	Diagnosis	Sample taken from	γ ATP-P/100 mg
1. 10 months	Hernia ing.	abdominal muscle	41
2. 20 »	Abscessus	the arm	95
3. 2 years	—	abdominal muscle	70
4. 2 »	Hernia ing.	»	50
5. 3 »	Retentio testis	»	50
6. 6 »	Hernia ing.	»	68
7. 7 »	—	»	53
8. 16 »	Appendicitis	»	72
9. —	—	»	70

Summary.

In the method for ATP determination from tissues we have presented above the same principle is followed as in the method for determining ATP from blood which we have published earlier; the specific enzyme obtained from potatoes splits off the end-phosphate group from the substrate molecule ATP, and the phosphate is measured photometrically.

At the same time the protein nitrogen determination is carried out from the precipitated protein with perchloric acid according to the micro-Kjeldahl method. The nitrogen content gives a statement of the size of the sample in question. We have given the results in γ ATP-phosphorus/100 mg protein.

References.

REHELL, B., O. FORSANDER and C.-E. RÄIHÄ, Scand. J. Clin. Lab. Invest. 1952. *4*. 211.
LEE, K. H. and J. J. EILER, Science 1951. *114*. 393.

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Studies on the Effect of Adrenaline and Noradrenaline on the Duodenal Secretion in Dog.

By

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No uniform behaviour can be demonstrated for the secretory elements of the digestive organs when these are subjected under the influence of adrenaline. The output of the salivary glands is increased by adrenaline administration (LANGLEY 1901, FLOROWSKI 1917, ALPERN 1925). The gastric acid output is claimed to be increased by adrenaline according to some students of the subject (YAKAWA 1908, LIM 1922, IVY and MCILVAIN 1923). Another group of investigators do not share this opinion (HESS 1920, ROTHLIN 1920, ALPERN 1923). The pancreas appears to be very sensitive for adrenaline. The effect is always a depressive (BENEDICENTI 1906, PEMBERTON and SWEET 1908, EDMUNDS 1910, MANN and MCLACHLIN 1917).

In the present investigation the effect of adrenaline and also of noradrenaline on the duodenal secretion was studied.

Methods and Material.

The experiments were carried out on 3 dogs prepared with duodenal pouches. The juice collected from these preparations presents mainly the output of the Brunner's glands. The operative procedures as well as the means for collection of the juice have been described in detail in previous papers (SONNENSCHEIN, GROSSMAN and IVY 1947, HARTIALA,

GROSSMAN and IVY 1950, HARTIALA, HAKOLA and TOIVONEN 1952). Also in the present study only the amount of juice was estimated.

The control secretory levels of the individual animals were established by daily observations on the secretion over a period of a month so that the mean secretory values given present the average of over 20 daily observations on each animal.

The experimental conditions were kept as constant as possible throughout the study. The dogs were fed their daily meals at the same hour of the day, they were taken to the laboratory always the same time in the morning etc. No other work was done in the same room, the same persons carried on all of the experiments. These precautions were made in order to avoid other, mainly psychic side-effects on the results. It is known that the Brunner's glands are under both nervous and humoral control (FLOREY and HARDING 1933 and 1934).

Results.

The experiments performed with adrenaline are recorded in table 1. The amount of single doses of the hormone corresponds to c. 0.025—0.040 mg per kg body weight. From the table it can be seen that there was no constant change in the duodenal secretion after adrenaline administration. In the majority of the experiments no change could be noted. In some of the experiments adrenaline injection was followed by increased duodenal secretion.

It is evident, however, that no rapid or short time changes in the secretion could be detected in this type of experimentation in which the observations were based on hourly collections of the juice. It was therefore reasoned to be worthwhile to carry on experiments with prolonged administration of the hormone. Intravenous infusions were given by means of a constant infusion pump. In these experiments 1.0 mg of the drug was administered during a 10 minutes period and 2.0 mg during a period of 50 minutes.

From table 1 it can be seen that the 1.0 mg dose (0.01 mg per kg body weight/minute) resulted in a nearly 100 per cent increase in the duodenal secretion in the 4 experiments performed this way. No effect could, however, be detected with the 2.0 mg dose (0.004 mg per kg body weight/minute) in two dogs (4 experiments) whereas one dog responded with a great increase.

From these experiments at least one conclusion may be drawn. Adrenaline when given either by a single injection or during a prolonged infusion has in no experiment caused a reduction in the secretion by the duodenal glands.

Table 1.

Effect of adrenaline on the duodenal secretion. The asterisk denotes the time of administration of the hormone. The control values represent the mean secretion of 21 observation days.

Dog	R					A					P				
	Volume of secretion (ml)					Volume of secretion (ml)					Volume of secretion (ml)				
	1st hour	2nd	3d	4th	5th	1st hour	2nd	3d	4th	5th	1st hour	2nd	3d	4th	5th
Control															
Mean	1.9	1.6	2.4	1.6	1.5	1.5	1.5	2.0	2.1	2.3	2.5	2.4	3.0	2.5	2.5
St. D.	.4	.4	.6	.6	.2	.4	.5	.4	.8	.8	.7	.9	1.0	.7	.8
Adrenaline			*					*			*				
0.25 mg	2.0	1.4	2.1	1.7	1.9	1.3	0.9	2.8	4.2	3.8	3.1	1.8	2.5	1.6	1.5
	*												*		
	1.3	1.6	2.6	1.8	1.4						3.6	3.4	3.8	3.3	3.8
	*							*			*				
0.40 mg	2.8	1.8	2.3	1.8	1.7	1.0	0.9	2.0	1.6	2.6	2.4	2.7	3.5	3.1	
	*					*									
	2.4	2.3	2.2	1.8	1.6	2.7	1.7	3.1	3.8	3.7					
1.0 mg during		*									*				
10 minutes	2.1	3.1	3.3	2.3	1.8						5.3	2.4	3.3	2.0	
	*										*				
	3.0	1.7	2.4	1.7							3.2	3.3	3.6	2.7	
2.0 mg during	*					*						*			
50 minutes	1.6	1.8	2.4	2.2	1.8	1.3	4.3	1.0	1.1	1.0	2.1	2.5	3.0	3.7	3.4
		*													
	1.3	1.3	2.2	1.4	2.4										
		*													
	1.4	1.5	2.0	1.0	1.3										

The experiments performed with noradrenaline are listed in table 2.

It was observed that only in one experiment out of 17 did noradrenaline cause a reduction in the duodenal secretion. This occurred in a dog who also showed rather great spontaneous fluctuations during the control period.

In the other experiments no response to noradrenaline was detected in 13 cases. In three experiments an increase in the secretion was noted, all in the same dog (R). The response of this animal did not differ from the other dogs in the adrenaline trials.

All the above mentioned remarks apply for the immediate response of the duodenal secretion to the tested adrenal hormones. In few experiments the infusion of adrenaline or noradrenaline resulted in a late-effect few hours after the administration. At this time a reduction in the secretion was observed.

Table 2.

Effect of noradrenaline on the duodenal secretion. The asterisk denotes the time of administration of the hormone. The control values represent the mean secretion of 21 observation days.

Dog	R					A					P				
	Volume of secretion (ml)					Volume of secretion (ml)					Volume of secretion (ml)				
	1st hour	2nd hour	3d hour	4th hour	5th hour	1st hour	2nd hour	3d hour	4th hour	5th hour	1st hour	2nd hour	3d hour	4th hour	5th hour
Control															
Mean	1.9	1.6	2.4	1.6	1.5	1.5	1.5	2.0	2.1	2.3	2.5	2.4	3.0	2.5	2.5
St. D.	.4	.4	.6	.6	.2	.4	.5	.4	.8	.8	.7	.9	1.0	.7	.8
Noradrenaline															
0.25 mg	* 2.2	1.8	2.5	1.9	1.5	* 0.8	2.0	1.4	2.3	3.8	* 1.9	1.7	2.3	2.8	2.5
	* 2.6	2.0	2.4	* 1.8		* 5.2	4.3	3.1	2.2	2.3	* 3.6		2.3	2.3	2.5
	1.7	* 1.3	* 2.3	1.5	1.4		*					*			
0.40 mg	* 1.9	1.9	2.3	1.9	1.7	1.0	1.4	1.3	1.5	1.2	2.5	2.7	2.6	2.8	1.9
	* 2.9	* 1.8	2.8	4.5	1.5										
	2.1	1.5	* 2.1	1.6	1.6							*			
1.0 mg during 10 minutes	2.2	2.0	4.0	5.1			*				2.3	2.7	2.8	2.7	2.3
2.0 mg during 50 minutes	* 1.5	1.4	2.2	1.3	1.9	* 1.0	2.0	1.2	0.9	0.9					
						1.5	2.2	1.3	1.2	1.4					

The juice secreted by the duodenal glands is in addition to the inorganic constituents composed mainly by highly viscous thick mucus. Although no direct analyses were performed the gross appearance of the juice did not change during the experimentation.

According to previous suggestions and experimental observations (Florey and Harding 1933 and 1934, Hartiala, Grossman and Ivy 1950, Hartiala and Grossman 1952) the duodenal glands seem to be of importance for the protection of the region against chemical irritants. It is interesting to note that these glands are not greatly affected by such changes in the blood supply of the intestinal mucosa which might be induced by adrenaline and noradrenaline.

At this point it should also be mentioned that according to Babkin (1950) adrenaline administration to dogs resulted in most cases in an increase of the spontaneous alkali and mucus secretion

by the stomach. BAXTER (1934) has observed that splanchnic nerve stimulation or injections of adrenaline gave the greatest amount of secretion in the pyloric part of the stomach, less in the body and still less in the fundus. Morphologically as well as functionally the pyloric glands are indistinguishable from the duodenal (Brunner's) glands.

Summary.

1. The effect of adrenaline and noradrenaline on the duodenal secretion was studied on dogs prepared with chronic duodenal pouches. The juice collected from these preparations presents mainly the mucus secretion of the Brunner's glands.

2. Single injections of adrenaline in doses of 0.025—0.040 mg per kg body weight resulted in half of the experiments in an increase of the duodenal secretion. Same doses of noradrenaline caused an increase in only 3 experiments out of 17. A depressive effect was observed only in one experiment.

3. Similar results were obtained by prolonged infusion of the hormones.

4. It was concluded that the physiologically important secretory output by the duodenal glands is not disturbed by the changes in the blood supply of the intestinal mucosa which might be induced by adrenaline and noradrenaline administration.

References.

ALPERN, D., Biochem. Z. 1923, *136*. 55.
— Pflüg. Arch. ges. Physiol. 1925. *299*. 723.
BABKIN, P. B., Secretory Mechanism of Digestive Glands. Paul Hoeber Inc. N. Y. 1950.
BAXTER, S. G., Amer. J. dig. Dis. 1934. *1*. 36.
BENEDICENTI, A., Arch. ital. Biol. 1906. *95*. 6.
EDMUNDS, C. W., J. Pharmacol. 1910. *2*. 559.
FLOREY, H. W., and H. E. HARDING, J. Path. Bact. 1933. *37*. 431.
— — Ibidem 1934. *39*. 255.
FLOROWSKI, G., Bull. Acad. Sci. (St. Petersburg) 1917. 119. Quoted by BABKIN.
HARTIALA, K. and M. I. GROSSMAN, J. Biol. Chem. 1952. *195*. 251.
— P. HAKOLA, and T. TOIVONEN, Ann. Med. Exp. Fenn. 1952. 30. 373.
— A. C. IVY, and M. I. GROSSMAN, Amer. J. Physiol. 1950. 162. 110.
HESS, W. R., Pflüg. Arch. ges. Physiol. 1920. *185*. 122.
IVY, A. C., and G. B. MCILVAIN, Amer. J. Physiol. 1923. *67*. 124.

LANGLEY, J. N., J. Physiol. 1901. *27*. 237.
LIM, R. K. S., Quart. J. Exp. Physiol. 1922. *13*. 79.
MANN, F. C., and L. C. MCLACHLIN, J. Pharmacol. 1917. *10*. 25.
PEMBERTON, B., and J. E. SWEET, Arch. int. Med. 1908. *1*. 628.
ROTHLIN, Communic. Intern. Physiol. Congr. 1920. Quoted by LIM.
SIROTININ, G. W., Z. ges. exp. Med. 1924. *40*. 90.
SONNENSCHEIN, R. R., M. I. GROSSMAN, and A. C. IVY, Acta Med. Scand. 1947. *196*. 295.
YAKAWA, G., Arch. Verdau Kr. 1908. *14*. 1908.

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Effect of Thermal Stress and Muscular Exercise, With and Without Insulin Hypoglycaemia, on the Body Temperature, Perspiration Rate, and Electrolyte and Lactate Content of Sweat.

By

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It is a common experience that prolonged physical effort, like long distance running or skiing, leads to exhaustion and ultimately to collapse probably caused by hypoglycaemia (SAVAGE and coworkers 1911, LEVINE, GORDON and DERICK 1924, SIPPE and BOSTOCK 1933). On the other hand, one of the main symptoms of hypoglycaemia in the resting organism is profuse perspiration (*e. g.* WRIGHT 1947). During hard muscular work the increased heat production is compensated by increasing the heat loss by sweating. As far as the writers know it has not been observed whether increased sweating caused by hypoglycaemia has any rôle in the causation of exhaustion. The increased loss of water and electrolytes and the decreased body temperature caused by profuse sweating could promote the appearance of exhaustion attacks. SAVAGE et al. (1911) observed that the body temperature of marathon runners, who had fallen out, was lower than that of those who had finished. On the other hand, it is a well known fact among sportsmen that a rapid decrease in sweating is one of the most severe complications of competitors, as it simultaneously leads to exhaustion (KUNNAS 1954).

Varying results have been observed concerning the lactate content of sweat. According to MCKEITH and his coworkers (1921)

Table

Effect of Hypoglycaemia on the Body Temperature, Sweating
N = 19;

	Blood Sugar mg %	Increase in Body Temperature °C	Decrease in Weight gram
Thermal Stress			
Hypoglycaemia	52.3 ± 16.4.	.0.82 ± 0.45	500 ± 130
Control	92.6 ± 8.6	1.20 ± 0.30	450 ± 190
Significance of the difference..		t = 2.988 0.01 > P > 0.001	
Muscular Exercise			
Hypoglycaemia	53.0 ± 13.9	0.98 ± 0.49	510 ± 290
Control	87.6 ± 12.8	1.30 ± 0.42	500 ± 140
Significance of the difference..		t = 2.156 0.05 > P > 0.01	

the increased lactate concentration of blood augments the lactate content of sweat. Recently, WEINER and HEYNINGEN (1952) have found that there is no connection between the lactate contents of blood and sweat.

In the present study the writers have examined the effect of hypoglycaemia on the rise in body temperature, on the perspiration rate, and electrolyte and lactate content of sweat produced by thermal stress as well as by muscular exercise.

Material and Method.

The material comprises 10 voluntary soldiers, all aged 20 years, and 9 students of physical education, aged 20—25 years. Hypoglycaemia was provoked by intravenous injections of regular insulin (Medica), in doses of 0.1 IU per kg of body weight. The same dose is used in the insulin tolerance test in clinical practice. In healthy persons this dose decreases the blood sugar to a half of its normal value in 20—30 min. (ALBRIGHT, FRASER and SMITH 1941). The actual experiment with collection of sweat was carried out 20 minutes after the injection. The sweating was induced in two ways, in the Finnish sauna-bath, in which the temperature varied from 65/48 to 90/45° C (dry bulb/wet bulb), and by running on a treadmill. The average distance run was 6,000 m, and the temperature of the room about 23/17° C. Sweat samples were collected in plastic sleeves, and the body weight and the rectal temperature measured using a common balance and clinical thermometer. The blood samples for glucose estimation were taken immediately before and after the experiment. The determination was made using

1.

Rate, and Electrolyte and Lactate Content of the Sweat.
Mean ± SD.

Sweating Rate in Sleeve ccm	Composition of Sweat			
	Na mEq./L	Cl mEq./L	K mEq./L	Lactate mg %
25.8 ± 10.2	55.7 ± 22.3	58.8 ± 17.6	8.12 + 1.40	198.6 ± 69.5
26.0 ± 10.3	69.2 ± 16.9	59.9 ± 28.2	7.84 ± 1.17	160.9 ± 63.0
				t = 1.756
				0.1 > P > 0.05
12.8 ± 6.1	60.9 ± 14.9	48.4 ± 13.1	9.44 ± 2.00	213.9 ± 83.2
13.9 ± 5.7	64.9 ± 20.2	49.2 ± 15.3	9.75 ± 6.90	188.6 ± 52.0
				t = 1.140
				0.3 > P > 0.2

the method of SOMOGYI (1945) and NELSON (1944). The average of the two determinations was chosen to indicate the level of blood sugar during the experiment. The chloride concentration of sweat samples was analysed titrimetrically using the method of SHALES and SHALES (1941) as modified by BRUN (1949). The determination of sodium and potassium was made using a flame photometer (KARVONEN and LAAMANEN 1954). The lactate estimation was carried out colorimetrically as described by FISTER (1950).

Parallel control experiments without injection of insulin on the same subjects were made.

Results.

The results are summarized in Table 1. It can be observed that the injections of insulin caused a significant decrease in the blood sugar concentrations. The rise in body temperature during hypoglycaemia was significantly smaller than during euglycaemia. On the contrary, there are no differences in the amount of collected sweat or in the total sweat rate identical with the decrease of weight. Nor are there any significant changes in the electrolyte content of sweat. In the sweat lactate concentrations there is some tendency to increased values with decreased blood sugar concentrations, but owing to the large individual variations these results revealed no significant difference. The individual changes in the rise of body temperature and in the lactate content of sweat are shown in Table 2. The individual rise of body temperature is strongly higher at the normal level of blood sugar than during

Table 2.

Differences in the Rises of Body Temperature and the Lactate Content of Sweat.

	Rise in Body Temperature °C	Rise in Lactate Content of Sweat mg %
Thermal Stress		
Significance of the rise	0.38 ± 0.47 $t = 3.524$ $0.01 > P > 0.001$	40.1 ± 84.9 $t = 2.045$ $0.1 > P > 0.05$
Muscular Exercise		
Significance of the rise	0.31 ± 0.52 $t = 2.610$ $0.05 > P > 0.01$	47.7 ± 103.6 $t = 1.780$ $0.1 > P > 0.05$

Table 3.

Differences in Sodium Content of the Sweat Between Soldiers and Students.
$N_1 = 10$, $N_2 = 9$; Mean $\pm$ SD.

	Thermal Stress	Significance of the difference	Muscular Exercise	Significance of the difference
In Hypoglycemia				
Soldiers	60.2 ± 13.4	$t = 1.145$	52.8 ± 6.8	$t = 3.006$
Students	71.8 ± 29.0	$0.3 > P > 0.2$	69.9 ± 16.5	$0.01 > P > 0.001$
Control				
Soldiers	62.3 ± 14.8	$t = 2.049$	53.3 ± 6.7	$t = 3.226$
Students	76.9 ± 16.5	$0.05 > P > 0.02$	77.7 ± 22.4	$0.01 > P > 0.001$

hypoglycaemia, but significant differences in the lactate content are not to be seen. In Table 3 the sodium concentrations of sweat between the groups of soldiers and students are compared. There are statistically significant differences in the sodium concentrations of these two groups. The soldiers have significantly lower sodium values in sweat produced by running than the students.

Discussion.

The level of blood sugar at which symptoms of hypoglycaemia appear varies individually. Most men present, however, classical symptoms with a blood sugar concentration of 50 to 60 mg per cent. Thus the decrease in blood sugar obtained in this work can be considered as a real state of hypoglycaemia.

Although a depression of blood sugar of the resting organism to an abnormally low level causes profuse sweating as one of its main symptoms, it does not have any effect on the sweating rate during thermal stress or muscular activity as shown in results presented above. The amount of sweat secreted from the skin area of one arm as well as the total amount of secreted sweat as indicated by decrease of weight seem to be quite independent of the blood sugar concentrations. From the point of view of this observation it is of great interest to notice the significantly smaller increase in body temperature during hypoglyceamia. It is not easy to find a reason for this relationship. It may be due to vasomotor changes produced by hypoglycaemia. The low blood sugar concentration may also decrease the metabolic rate. The distribution of sweating might be affected by injection of insulin. Further studies are necessary to make these questions clear.

From the sports-physiological point of view it is of great interest that hypoglycaemia fails to have any effect on the electrolyte content of sweat. Nor has it any significant effect on the lactate secretion.

As pointed out by CONN and his coworkers (1948), the sodium concentration of sweat is a valuable index of the adrenocortical activity. Low values of sodium indicate increased adrenocortical function. The sodium values of the soldiers' group obtained in the present investigation agree well with those obtained by AHLMAN and coworkers (1953). The values of the students' group seem, on the contrary, relatively high. It may indicate that this group was in a state of adrenocortical insufficiency caused by overtraining.

Summary.

Effect of thermal stress (Finnish Sauna-bath) and muscular exercise (on treadmill) on the rise in body temperature, on the sweating rate, and electrolyte and lactate content of sweat is studied. In one half of the experiments, hypoglycaemia was induced by intravenous injections of insulin. There are no significant differences in the rate of sweating and in the composition of sweat during insulin hypoglycaemia and during euglycaemia. In the lactate content of sweat there is some tendency to increased values with decreased blood sugar concentrations. The rise in

body temperature under insulin hypoglycaemia is significantly lower than in experiments without insulin injection. The reason for this observation is briefly discussed.

References.

AHLMAN, K. L., O. ERÄNKÖ, M. J. KARVONEN and V. LEPPÄNEN, J. Clin. Endocr. 1953. *8*. 773.

ALBRIGHT, F., J. R. FRASER and P. H. SMITH, Ibidem 1941. *1*. 300.

BRUN, C., Nord. med. 1949. *42*. 1774.

CONN, J. W., L. H. LOUIS and M. W. JOHNSTON, J. Clin. Invest. 1948. *27*. 529.

FISTER, H. J., Manual of Standardized Procedures for Spectrophotometric Chemistry, Standard Scientific Supply Corporation, New York 1950.

KARVONEN, M. J. and A. LAAMANEN, to be published 1954.

KUNNAS, M., personal communication 1954.

LEVINE, S. A., B. GORDON and C. L. DERICK, J. A. M. A. 1924. *82*. 1778.

MCKEITH, N. W., M. S. PEMBREY, W. R. SPURRELL, E. C. WARNER and H. J. W. J. WESTLAKE, J. Physiol. 1921. *55*. VI.

NELSON, N., J. Biol. Chem. 1944. *153*. 375.

SAVAGE, W. L., E. S. DICKEY, C. B. MCABOY, C. ORBIN and A. W. WOODBURG, Amer. Phys. Educ. Rev. 1911. *16*. 1.

SCHALES, O. and S. S. SCHALES, J. Biol. Chem. 1941. *140*. 879.

SIPPE, C. and J. BOSTOCK, M. J. Australia 1933. *1*. 207.

SOMOGYI, M., J. Biol. Chem. 1945. *160*. 69.

WEINER, J. S. and R. E. VAN HEYNINGEN, J. Appl. Physiol. 1952. *4*. 734.

WRIGHT, S., Applied Physiology, Eighth Edition, Oxford Medical Publications, London 1947.

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Metabolic Responses of Human Subjects to Severe Acute Thermal Stress.

By

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The physiological reactions of animals subjected to various environmental temperatures have been studied very extensively. Most of these studies, however, deal with temperatures that differ only slightly from the comfort zone. The metabolic rate (MR) of the rabbit during thermal stress has been studied by PFLÜGER (1878), who found that a rise in the MR took place with rising body temperature. Hot baths were found to cause a rise as well in body temperature as in MR of man (KOEHLER 1923, LANDIS and al. 1926). PLAUT and WILBRAND (1922), using an electric light-arch as a heat source, observed that the MR was increased by increased blood temperature. MCCONNELL and YAGLOGLOU (1924, 1925), LUNDGREN (1933), and OTT (1948) have also found an increase in MR on exposure to high ambient temperatures.

Observations on MR after thermal stress, on the other hand, are not equally numerous. PLAUT and WILBRAND state that 2—3 hours after heating, when the pulse rate, respiration, and body temperature have become normal, there is a decrease in the MR of the guinea pig, of the dog, and of man. LUNDGREN has studied the MR of man 15 and 45 minutes after a Finnish Sauna-bath and found values somewhat lower than those preceding the bath. OTT has measured the MR 0.5, 1, and 24 hours after the Sauna-bath; at 60 minutes and 24 hours after the Sauna-bath some values are slightly lower than before the Sauna, but

the averages are above the starting level. Most interesting in this respect are the findings of MANSFELD (1949), who states that thermal stress causes the secretion of a thyroxine antagonistic, metabolism lowering hormone (thermothyrin A) into the blood stream. SUOMALAINEN and MÄKIPAJA (1951, 1952) have claimed that such a metabolism lowering factor can be found in the serum of man after a Sauna-bath. NIEMI (1953), on the other hand, has not been able to confirm the occurrence of thermothyrin A after Sauna-bath.

The purpose of this work has been to study a) the changes in the MR of man brought about by high environmental temperature, b) changes in the MR after a thermal stress, and c) possible correlations between MR, rectal temperature, skin temperature, and pulse rate.

Material and Methods.

The material consisted of 16 healthy students, whose ages varied between 15 and 24 years. Of these 8 were women and 8 men. Two series of experiments were carried out. 1) Subjects 1—10 — 5 women and 5 men — lived for 24 hours on a diet poor in protein. In the laboratory they took off their clothes and rested for about half an hour on a comfortable bed, clothed in a light laboratory coat, after which two successive MR (oxygen consumption) measurements were made with a Krogh closed circuit respirometer. Between the mouthpiece and the original respirometer a rubber tubing of about 30 cms length was attached, which was needed for making measurements during the heat stress (see below). In control experiments this additional dead space was not found to affect the results. Immediately after each MR measurement the pulse rate, skin temperature, and rectal temperature were recorded; the same recording order was followed during all phases of the experiment. The skin temperature was measured from the skin above the sternal manubrium with a thermocouple (manufactured by Elektrolaboratoriet, Copenhagen), the rectal temperature with a clinical thermometer. After these two measurements the subjects walked to the Sauna-bath in the adjoining room (a walk of about 10 meters), which was used for producing the high temperature. The air temperature in the Sauna varied during different experiments between 55° C D. B./36° C W. B. and 71° C/39° C; the temperature during each experiment was kept approximately constant.

The respirometer was connected to the Sauna with the rubber tubing mentioned above, which was inserted through a hole in the wall. In the Sauna the subjects lay naked on a wooden bench for about 40 minutes, during which time 2 measurements were made. After coming from the Sauna the subjects dried themselves without washing, put on the laboratory coat, and walked back to the original dressing room, where

they lay down on the bed. After some minutes the recording was begun again. After the Sauna-bath 4 recordings were made, with 15—20 minutes intervals.

Control experiments consisted of a procedure identical with that of the original experiment, except that the Sauna was not heated.

2) Subjects 11—16. The MR was measured in the morning, and on the same evening the subjects went into the Sauna-bath. In the Sauna the subjects sat or lay naked on wooden benches. They did not stay in the Sauna-bath continuously, but were allowed to come out for a while if they felt too hot. The effective time in the Sauna-bath was about 15 minutes for the women and 20—35 minutes for the men. After the Sauna-bath the subjects washed themselves with warm water. The following morning, 10 hours after the thermal stress, the MR was measured again; recordings of the MR of the men were also made 18 hours after the Sauna-bath.

In the control experiments a warm shower was taken instead of the Sauna-bath.

In all, 95 recordings were made, not including the control experiments. 1 MR measurement (of subject 8) could not be obtained because of nausea, and 2 MR recordings (of subjects 4 and 6) were discarded because of technical errors.

For the basal pre-heating value of the MR the average of the two first measurements was taken, if they did not differ more than 0.3 l. If this was the case, the lower of the two was held to be the more accurate one. For the pre-heating value of the rectal and skin temperatures and the pulse rate the average of the two was considered to be the right one.

Accuracy of Methods.

The Krogh apparatus is generally considered to be moderately reliable. According to KARVONEN and NIEMI (1952), the methodical error is less than 7 %. During the experiment the subjects grew a little restless and showed signs of boredom. The effect of successive determinations on the MR-results have been studied quite extensively, but the results are contradictory (BOOTHBY and SANDIFORD 1922, SHOCK 1942, VOGELIUS 1945, DAHLSTRÖM 1950, LAROCHE and RICHET 1950, PEELER et al. 1950, KARVONEN and NIEMI 1952). In our control experiments the average values were alternately high and low and varied between +2 and +8 %. The standard deviation was very great.

Calculations showed that the termometrical errors in rectal temperature measurements due to the high environmental temperature were negligible. MEAD and BONMARITO (1949) have discussed the rectal temperature measurement in general, and state that rectal readings are not always a good index of the body temperature. It is possible that the values obtained are not absolute, but we believe they are a relatively good index of the changes in the mean body temperature.

Of all the measurements made, the skin temperature readings are the least reliable ones. STOLL and HARDY (1950) have criticized the

thermocouple methods for measuring skin temperature and claim that the results are affected by the pressure with which the thermocouple is applied to the skin among other factors. This seems reasonable enough. No mechanical devices for applying the thermocouple was used in the present work; it was mounted on a plastic handle by which it was gently pressed on the skin. Variations in the application pressure were of course uncontrollable and the writers had to rely on subjective judgement. One could expect that the thermocouple readings in the Sauna-bath would be grossly affected by the high air temperature. On the surface of the body, however, there exists a "personal atmosphere", a layer of still air of considerable thickness (FOURT and HARRIS 1949), which eliminated extensively the effect of heat convection from the hot environment.

On the whole the methods seem to be, if not absolutely reliable, at least sufficient to give good relative readings of the MR, body temperature and skin temperature.

Results.

The results from series 1 are somewhat variable (Fig. 1). The MR is expressed as per cent over or below the pre-heating value. 6 of the 10 subjects reacted quite uniformly to the sudden rise in environmental temperature and the fall following it. When entering the Sauna-bath the MR, rectal temperature, skin temperature, and pulse rate of the subjects all showed a distinct rise. After 20—30 minutes' sojourn in the hot environment they reached the average values of about +20 %, 38.3° C, 38.9° C, and 101/min. When normal conditions were restored, the values showed a decrease which was much slower than the rise. 60—70 minutes after the thermal stress the MR was on the average still increased, and so were the rectal and skin temperatures. The pulse rate, however, returned to the pre-heating level 20—30 minutes after the exposure.

The four remaining subjects, on the other hand, showed quite contradictory reaction patterns. There was an enormous rise (about 60 %) in the MR of one subject (No. 4, male) when entering the hot atmosphere, with a subsequent fall during the following 20 minutes of heat stress. Two other subjects (Nos. 5, female, and 7, male), on the contrary, reacted with a sudden decrease in the MR when entering the hot room. Toward the end of the heating their MR then increased, but remained after it on a slightly sub-basal level. In the last case (subject 10, male) there occurred a slight fall in the MR (about —8 %), followed by a typical rise and by a subsequent fall after heating.

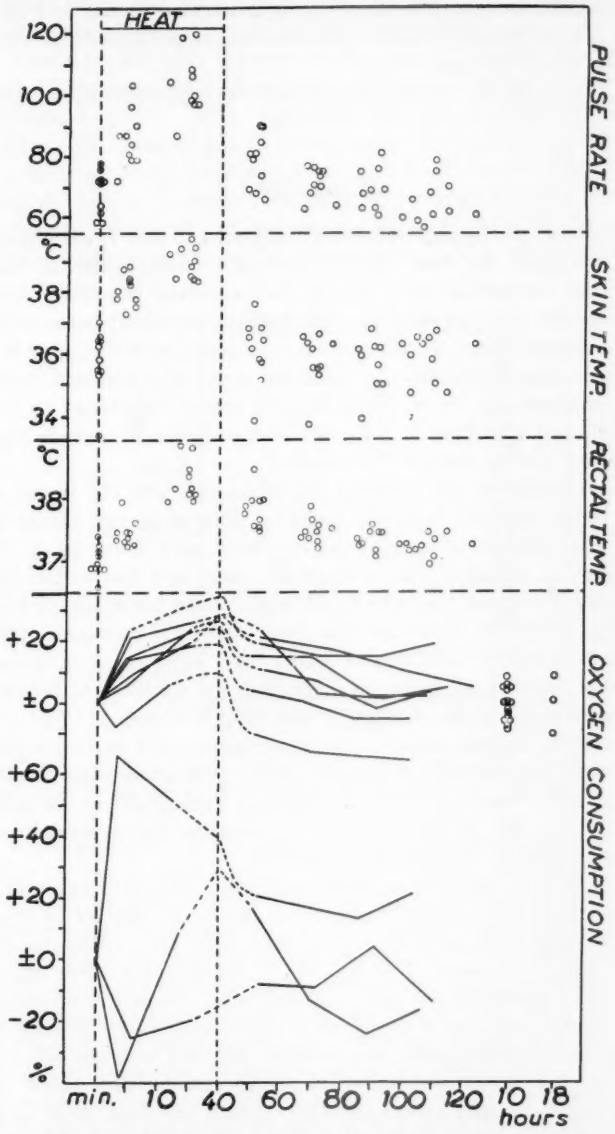

Fig. 1. Pulse rate, skin temperature, rectal temperature, and oxygen consumption (expressed in beats/minute, °C, and percents over or below the pre-heating value, respectively) during and after severe thermal stress. The pre-heating values of the pulse rates and skin and rectal temperatures are also shown. Upper oxygen consumption curves: subjects 1—3, 6, and 8—10, lower: subjects 4, 5 and 7. Circles: subjects 11—16. Time in minutes from the beginning of the thermal stress. Dotted lines: estimated courses of the metabolic rates.

The MRs of the subjects of series 2 are also shown in Fig. 1. The MR at 10 and 18 hours after thermal stress showed no alterations from the basal level.

In the control experiments no uniform tendencies were found.

Discussion.

Three of the atypical reactions (subjects 4, 5 and 7) are obscure and we have not been able to interpret them. Even on close scrutiny no methodical errors in the recording procedure could be detected. The sudden encounter with the extremely hot environment almost certainly affected the subjects mentally, and it is possible that these strange reactions could be explained rather on an emotional basis. There are no concrete facts as to that point, and therefore we have found it suitable to exclude these results from the further discussion.

The fourth of the atypical reactions (subject 10) is not so markedly different from the majority. Except for the initial fall the MR follows the general pattern, only on a lower level. The subject in question was a muscular man and the initial pre-heating MR value was very much higher than his standard BMR value calculated from the Du Bois scales. Hindmarsh (1927) and Pickworth (1927) have shown that a decrease in normal muscle tone of subjects, accomplished by training, is followed by a decrease of about 20 % in the MR. Hindmarsh (1927) and Lee (1940) suggest that in a warm environment a more complete relaxation of skeletal muscle takes place, with a following decrease in the MR. This seems to us a very good explanation of the initial fall in the MR of this subject. On repeating the experiment an exactly similar reaction was obtained.

The typical rises are in good conformity with previous results. Lundgren (1933) found that an increase in the MR of 24 % on the average took place during a Sauna-bath. Ott (1948) observed an average increase of about 21 % immediately after Sauna. In our subjects the average increase after about 30 minutes in the Sauna was about 20 %. 60 minutes after the thermal stress both the average rectal temperature and the average MR were above the initial level, and no significant decrease below the pre-heating values occurred either during the first hour or 10 and 18 hours after the heating. The sub-basal findings of Lund-

GREN (1933) and OTT (1948) are few and mostly within the methodical error.

This does not give support to the results obtained by PLAUT and WILBRAND (1922), MANSFELD (1949), and SUOMALAINEN and MÄKIPAJA (1951, 1952), but confirms the findings of NIEMI (1953). The heat stress is obviously severe enough to elicit a counter-regulatory reaction predicted by MANSFELD (1949), so that the failure to observe anything of the kind strongly requires further critical study of the thermothyrin subject. The hitherto published results are rather contradictory (*e. g.* BERDE (1948), VAN GOOR (1951)).

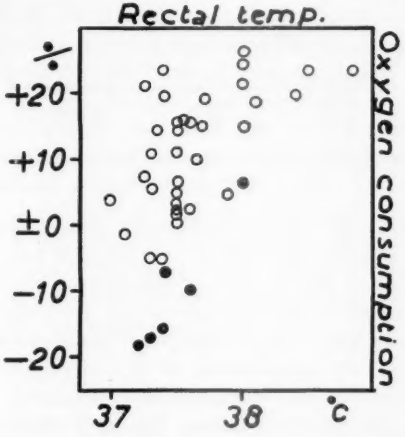

Fig. 2. Correlation between rectal temperature and oxygen consumption (subjects 1—3, 6 and 8—10). The values expressed as in Fig. 1. Dots: the values of subject 10.

In Figures 2, 3 and 4 the MR is plotted against the simultaneous body temperature, skin temperature, and pulse rate respectively. PFLÜGER (1878) found that a rise of 1° C in the body temperature caused a rise of about 5.7 % in the oxygen consumption of the rabbit. From the results of MCCONNELL and YAGLOGLOU (1924, 1925) it appears that the heat production of man increased about 40 % when the body temperature had risen by 1° C. This increase in the MR was accompanied by an increase in the pulse rate by about 40 beats/min. CHRISTENSEN (1938) states that in hyperthermia induced by diathermy a rise in the body temperature of 1° C was followed by an increase of 10.8 % in the MR. DU BOIS

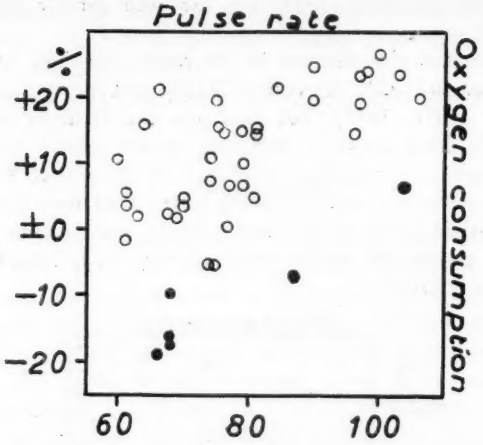

Fig. 3. Correlation between skin temperature and oxygen consumption. The explanations as in Fig. 2.

(1921) found that in active hyperthermia (fever), when the body temperature had been elevated by 1° C, the MR had increased by about 13 %. It is apparent from the figures that also in our results there exists a positive correlation between the MR on one side and the body temperature, skin temperature, and pulse rate. A rise in body temperature of 1° C corresponds to about 17 % increase in the MR, which is almost accurately the average of the previously reported values. The skin temperature is increased about 2.5° C and the pulse rate about 25 beats/min.

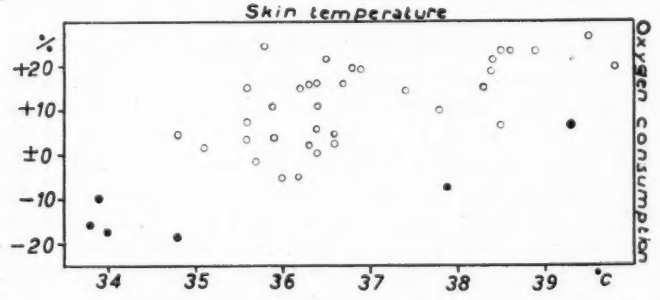

Fig. 4. Correlation between pulse rate and oxygen consumption. The explanations as in Fig. 2.

These are average values and the standard deviation is quite great, so that the calculation of exact correlation coefficients does not seem justified.

In conclusion it can be said that when subjecting man to very high environmental temperatures some atypical reactions can be found as in most extreme conditions, while the majority follows a typical pattern with slight individual variations.

The rise in the MR during thermal stress may or may not be due only to the accelerating effect of rising temperature on the chemical reactions in the organism — the increased activity of the sweat glands and respiratory muscles does not account for the whole rise in the MR (PLAUT and WILBRAND (1922), CHRISTENSEN (1938)) — whatever the cause, the reaction itself seems highly unsuitable for the thermal homeostasis of the body. A counter-regulatory mechanism could therefore be supposed to exist. We have, however, not been able to demonstrate any such mechanism in man, the effects of which could be observed even after the thermal stress. MANSFELD (1949) and others have published an abundance of data demonstrating the existence of thermothyrin A, but the discrepancies between these results and other investigations (VAN GOOR (1951), NIEMI (1953), the present study) makes the secretion of thermothyrin A a highly questionable function in the human organism.

Summary.

1. The oxygen consumption, rectal temperature, skin temperature, and pulse rate of human subjects during and after exposure to extremely high environmental temperatures have been recorded.

2. A rise in the metabolic rate during thermal stress was observed, with a subsequent fall after it. No decrease in the metabolic rate below the pre-heating level could be observed during the first hour, 10 hours, and 18 hours after the thermal stress.

3. Positive correlations between the metabolic rate and the rectal temperature, skin temperature, and pulse rate were observed.

References.

BERDE, B., Experientia 1948. *4*. 231.
BOOTHBY, W. M. and I. SANDIFORD, J. Biol. Chem. 1922. *54*. 767.
CHRISTENSEN, E. H., Arbeitsphysiol. 1933. *7*. 120.

DAHLSTRÖM, H., Acta Physiol. Scand. 1950. *21.* Suppl. 71.
DU BOIS, E. F., J. A. M. A. 1921. *77.* 352.
FOURT, L. and M. HARRIS, Physiology of Heat Regulation and The Science of Clothing. Edited by NEWBURGH, L. H., W. B. Saunders Company, Philadelphia 1949.
VAN GOOR, H., Acta physiol. pharm. Neerl. 1951. *1.* 525.
HINDMARSH, E. M., Aust. J. Exp. Biol. Med. Sci. 1927. *4.* 225.
KARVONEN, M. J. and M. NIEMI, Scand. J. Clin. Lab. Invest. 1952. *4.* 112.
KOEHLER, A. E., Arch. Int. Med. 1923. *31.* 590.
LANDIS, E. M., W. L. LONG, J. W. DUNN, C. JACKSON and U. MEYER, Amer. J. Physiol. 1926. *76.* 35.
LAROCHE, G. and C. RICHET, Pr. Méd. 1950. *58.* 1253.
LEE, D. H. K., A Basis for the Study of Man's Reaction to Tropical Climates. Brisbane, Aust., U. of Queensland, 1940. (Cited from ROBINSON, S. in Physiology of Heat Regulation and The Science of Clothing. Edited by Newburgh, L. H., W. B. Saunders Company, Philadelphia 1949.)
LUNDGREN, R., 1933 Thesis. Kouvola (Finland).
MANSFELD, G., The Thyroid Hormones and their Action, Frederik Muller Ltd. London 1949.
MCCONNELL, W. J., C. P. YAGLOGLOU and W. B. FULTON, U. S. Pub. Health Rep. 1924. *39.* 3075.
MCCONNELL, W. J., C. P. YAGLOGLOU and W. B. FULTON, Arch. Int. Med. 1925. *36.* 3.
MEAD, J. and C. L. BONMARITO, J. Appl. Physiol. 1949. *2.* 97.
NIEMI, M., Acta Physiol. Scand. 1953. *28.* 172.
OTT, V. R., Die Sauna, Benno Schwabe & Co. Basel 1948.
PEELER, A. L., O. E. HEPLER, V. M. KINNEY, L. E. CISLER and F. T. JUNG, J. Appl. Physiol. 1950. *3.* 197.
PFLÜGER, E., Arch. Physiol. 1878. *18.* 247.
PICKWORTH, F. A., Proc. Roy. Soc. 1927. *101.* 163.
PLAUT, R. and P. WILBRAND, Z. Biol. 1922. *74.* 191.
SHOCK, N. W., Amer. J. Dis. Child. 1942. *64.* 19.
STOLL, A. M. and J. D. HARDY, J. Appl. Physiol. 1950. *2.* 531.
SUOMALAINEN, P. and E.-L. MÄKIPAJA, Experientia 1951. *7.*
SUOMALAINEN, P. and E.-L. MÄKIPAJA, Arch. Soc. Zool. Bot. Fenn. 'Vanamo' 1952. *6.* 2.
VOGELIUS, H., Acta med. Scand. 1945. Suppl. 165.

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Rabbit Red Cell Haemolysing Property of Human Plasma.

By

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When investigating the haemopoietic activity of plasma by means of injecting it into test animals of another species one is confronted with the difficulty that the haemopoietic activity of the injected plasma may be confused because of a haemolysing or agglutinating effect on the recipient's blood cells (BONSDORFF and JALAVISTO 1948). The aim of the present paper is to study the rabbit blood haemolysing property of human plasma and search for some means to abolish or control the effect.

The occurrence of natural haemolysins and agglutinins in the serum of some animals against the red cells of other species has been established by several investigators (EHRLICH and MORGENROTH 1899, LOEB and co-workers 1910, STUART and co-workers 1935, SIEVERS 1937, DAVIDSOHN and STERN 1949 and 1950, and others). Natural as well as immune haemolysins have been demonstrated also in vitro, though the effect of immune haemolysins, which have been the subject of more investigation, is claimed to be stronger in vivo (WASASTJERNA 1948 and 1951, CASTLE and co-workers 1950).

Literature knows several ways to abolish the haemolytic activity of foreign plasma: *e. g.* to absorb the haemolysins into the red cells or organ extracts of the recipient animal (SIEVERS 1937, DAVIDSOHN and STERN 1949 and 1950), or to immunize the recipient animal by means of small, repeated injections of the donors plasma (LOEB and co-workers).

In the present study the following questions were studied:

Haemolysing effect of human plasma on rabbit blood in vivo and in vitro.

Effect of incubation with rabbit blood cells on the haemolytic activity of human plasma in vitro.

Effect of immunization by repeated injections of human plasma on the susceptibility of rabbit blood to undergo haemolysis as a result of human plasma injection.

Methods.

The technique used differs from the usual techniques of serological studies, as not the free haemoglobin in plasma, but the decrease of the red cells in the recipient's blood is considered as the criterion of haemolysis. This technique is chosen for practical reasons, for when investigating the haemopoietic activity of a plasma sample the interesting question is whether the plasma causes a decrease of the recipient's peripheral red cell count, and not whether the decrease is called forth purely by haemolysis or haemolysis preceded by or combined with intravascular agglutination of the red cells. In experiments in vivo there still remains the possibility that the decrease in the red cell count is partly due to haemodilution, and thus could not be controlled by controlling the haemolytic reactions. This, however, is not likely as the injected amounts of plasma are small: even much larger amounts of homologous plasma are claimed to cause no noticeable change in the peripheral erythrocyte count (ERSLEY 1953).

The experiments were carried out as follows:

Haemolysis in vitro: Samples of rabbit blood were drawn from the marginal ear vein or from the heart, using heparin as anticoagulant, and the red cells were enumerated from the samples in a BUERKER counting chamber. The blood samples were then divided into two parts of 1 cc; into the first part 0.01 cc of physiological saline or heparinized "absorbed" human plasma, and into the second part 0.01 cc of heparinized untreated human plasma was added. The samples were then incubated at 38—40° C for 1 hour, and the erythrocytes were enumerated from each sample after careful mixing. For absorption of human plasma into rabbit blood cells a heparinized sample of rabbit blood was centrifuged, the separated cells were washed once with physiological saline, and diluted or undiluted heparinized human plasma was added in proportion 0.1 cc of undiluted plasma to 1 cc of cells. Absorption took place at a room temperature of 20—22° C, and for 20 to 45 minutes.

To test the haemolytic activity of human plasma on rabbit blood in vivo 1.0—1.5 cc of heparinized fresh human plasma was injected into the ear vein of the rabbits. The recipient's red cells were enumerated before the injection and after 1 hour.

Immunization against human plasma was carried out by injecting increasing amounts (0.8—1.8 cc) of heparinized human plasma into the

ear vein of the rabbits. In the first experimental series (effect of human plasma on rabbit blood in vitro) only 2 or 3 injections were given, while in the last series (effect of immunization on the susceptibility of rabbit blood to undergo haemolysis after injection of human plasma) several injections were given, until there was no more haemolytic reactions after the injection.

Results.

Effect of human plasma on rabbit blood in vitro. — Blood samples from 22 untreated rabbits were incubated at 38—40° C for 1 hour with human plasma and with physiological saline simultaneously. The red blood cells were counted before and after the incubation. It was observed that after incubation with saline there was no or only a slight decrease in the number of red cells, whereas after incubation with plasma there usually was a more marked decrease. The results are summarized in table 1. It is seen, that the difference between the erythrocyte count in the "saline" and "plasma" tube, though slight, is rather constant, and statistically significant ($P < 0.001$).

A second series of blood samples from 9 rabbits, which had previously been given 2—3 injections of human plasma were incubated with addition of human plasma and saline. In this group, as in the group of untreated rabbits, there was no or a very slight decrease of the red cell count in the "saline" tube, and, in most cases, a slightly more pronounced decrease in the "plasma" tube (table 2). The average difference between the "plasma" and "saline" tube, however, is here slighter than in the group of untreated rabbits, and statistically only probable ($0.02 < P < 0.05$). If the difference of the average decrease in the "plasma" tubes in the untreated and immunized group is calculated, a value bordering on statistical probability is obtained. These results suggest that while 2—3 injections of human plasma as a rule decrease the susceptibility of rabbit blood cells to undergo haemolysis in the presence of human plasma, the treatment still yields no certain guard against the haemolysing effect of human plasma.

To test the effect of incubation with rabbit blood cells on the ability of human plasma to haemolyse rabbit red cells, 8 samples of rabbit blood were divided into two parts; into the one part untreated human plasma, and into the other part "absorbed" human plasma was added. After incubation for 1 hour at 38—40° C the number of erythrocytes in the "absorbed" plasma tube

Table 1.

Effect of incubation with human plasma on the blood of untreated rabbits.

Rabbit	Plasma group	Initial Er count mill./cu.mm	Deviation from the initial Er count after incubation		Difference
			with saline mill./cu.mm	with plasma mill./cu.mm	
1.....	AB	5.0	— 0.1	— 0.4	— 0.3
2.....	AB	5.2	— 0.1	— 0.6	— 0.5
3.....	AB	4.8	— 0.1	0.0	+ 0.1
4.....	O	5.1	+ 0.1	— 0.3	— 0.4
5.....	O	5.5	— 0.1	— 0.4	— 0.3
6.....	AB	4.8	0.0	— 0.2	— 0.2
7.....	A	5.1	— 0.1	— 0.6	— 0.5
8.....	A	4.6	— 0.2	— 0.6	— 0.4
9.....	A	4.4	— 0.1	— 0.5	— 0.4
10.....	O	4.7	+ 0.2	— 0.3	— 0.5
11.....	O	4.7	— 0.1	— 0.6	— 0.5
12.....	AB	4.6	— 0.3	— 0.3	0.0
13.....	AB	4.9	— 0.4	— 0.4	0.0
14.....	O	5.4	0.0	0.0	0.0
15.....	A	6.2	— 0.3	— 0.4	— 0.1
16.....	AB	5.5	0.0	— 0.1	— 0.1
17.....	A	5.3	— 0.2	— 0.1	+ 0.1
18.....	A	5.3	— 0.3	— 0.5	— 0.2
19.....	A	5.3	— 0.3	— 0.5	— 0.2
20.....	A	5.3	— 0.1	— 0.4	— 0.3
21.....	A	5.3	— 0.1	— 0.4	— 0.3
22.....	A	5.3	— 0.1	— 0.5	— 0.4
Mean		5.08 ± 0.084	— 0.12 ± 0.025	— 0.37 ± 0.038	— 0.25 ± 0.046

Table 2.

Effect on incubation with human plasma on the blood of immunized rabbits.

Rabbit	Plasma group	Initial Er count mill./cu.mm	Deviation from the initial Er count after incubation		Difference
			with saline mill./cu.mm	with plasma mill./cu.mm	
1.....	A	5.1	— 0.1	0.0	+ 0.1
2.....	O	4.9	— 0.1	— 0.1	0.0
3.....	O	4.4	0.0	— 0.2	— 0.2
4.....	O	5.1	0.0	— 0.2	— 0.2
5.....	AB	4.7	+ 0.1	— 0.2	— 0.3
6.....	AB	4.7	— 0.2	— 0.5	— 0.3
7.....	A	4.9	— 0.1	— 0.2	— 0.1
8.....	O	5.4	0.0	— 0.1	— 0.1
9.....	O	5.1	— 0.1	— 0.0	0.0
Mean		4.92 ± 0.095	— 0.06 ± 0.028	— 0.18 ± 0.038	— 0.12 ± 0.047

Table 3.

Effect of incubation with untreated and "absorbed" human plasma on rabbit blood.

Rabbit	Plasma group	Decrease in the Er count after incubation with		Difference
		untreated plasma mill./cu.mm	absorbed plasma mill./cu.mm	
1.....	AB	— 0.4	— 0.1	— 0.3
2.....	A	— 0.2	+ 0.1	— 0.3
3.....	O	— 0.2	+ 0.1	— 0.3
4.....	O	— 0.2	— 0.1	— 0.1
5.....	O	— 1.0	— 0.3	— 0.7
6.....	AB	— 0.1	— 0.1	0.0
7.....	AB	— 0.4	— 0.6	+ 0.2
8.....	AB	— 0.1	+ 0.1	— 0.2
Mean		— 0.32 ± 0.089	— 0.11 ± 0.078	— 0.21 ± 0.085

was only slightly decreased, whereas there was a marked decrease in the "untreated" plasma tube (table 3). When the average difference of erythrocyte count in the two tubes is calculated, a value indicating statistical probability is obtained ($0.02 < P < 0.05$), *i. e.* it seems probable that incubation with rabbit cells diminishes the ability of human plasma to haemolyse rabbit red cells.

In the following experimental series the rabbit red cell haemolysing activity of human plasma in vitro and in vivo was compared. 11 untreated rabbits were given injections of 1.0—1.5 cc of human plasma, and simultaneously blood samples taken previous to the injection were incubated with human plasma, the ratio human plasma/rabbit cells being approximately the same in vivo and in vitro. The results (table 4) suggest that there is some correlation between the haemolytic reaction in vivo and in vitro, for in cases where there is no reaction in vitro the reaction in vivo often is small or absent, and when the reaction in vivo is very strong there is haemolysis also in vitro. However, the average decrease in vitro is not as great and as constant as in vivo. Thus, the haemolytic effect of human plasma injection in vivo can be evaluated by means of an in vitro test with some probability, but not with certainty.

The effect of repeated injections on the susceptibility of rabbit blood to undergo haemolysis after injection of human plasma was investigated in 3 rabbits which initially showed haemolysis after human plasma injection. These experiments are represented in

Table 4.

Comparison of the rabbit red cell haemolysing activity of human plasma in vivo and in vitro.

Rabbit	Plasma group	In vivo: Deviation from the initial value 1 hour after injection mill. Er/cu.mm	In vitro: Deviation from the initial value after incubation with saline mill. Er/cu.mm	In vitro: Deviation from the initial value after incubation with plasma mill. Er/cu.mm	Difference
1	A	— 0.7	— 0.4	— 0.3	+ 0.1
2	A	— 0.6	— 0.3	— 0.4	— 0.1
3	A	— 0.3	0.0	— 0.1	— 0.1
4	AB	0.0	— 0.4	— 0.4	0.0
5	A	— 0.7	— 0.2	— 0.1	+ 0.1
6	A	— 0.9	— 0.2	— 0.6	— 0.4
7	A	— 0.6	— 0.1	— 0.6	— 0.5
8	O	— 0.2	+ 0.2	— 0.3	— 0.5
9	AB	— 0.1	— 0.3	— 0.3	0.0
10	AB	— 0.5	— 0.2	— 0.7	— 0.5
11	AB	— 0.5	— 0.1	— 0.7	— 0.6
Mean		— 0.46 ± 0.084	— 0.18 ± 0.049	— 0.41 ± 0.066	—0.23±0.094

Table 5.

Effect of repeated injections of human plasma on the susceptibility of rabbit blood to haemolyse after human plasma injection.

Date of injection	Plasma group	Initial Er value mill./cu.mm	Er 1 hour after injection mill./cu.mm	Difference
Rabbit 1.				
23. 10. 53	AB	5.0	4.5	— 0.5
27. 10. »	AB	5.0	4.3	— 0.7
31. 10. »	AB	4.4	4.0	— 0.4
4. 11. »	AB	4.5	4.3	— 0.2
17. 11. »	AB	5.0	5.0	0.0
12. 12. »	A	4.7	4.7	0.0
Rabbit 2.				
27. 11. 53	AB	5.4	4.8	— 0.6
1. 12. »	O	4.2	4.3	+ 0.1
12. 12. »	A	4.8	4.7	— 0.1
16. 12. »	AB	4.5	4.6	+ 0.1
Rabbit 3.				
14. 12. 53	A	5.0	4.4	— 0.6
17. 12. »	A	4.6	4.1	— 0.5
22. 12. »	A	4.6	4.5	— 0.1
8. 1. 54	A	4.2	4.2	0.0

table 5. It is seen that in each rabbit fair immunity was obtained, though in one case several injections were needed (rabbit 1). Control experiments after 2—4 weeks show that immunity was preserved for this period. Accordingly, it seems that the property of rabbit red cells to haemolyse after injection of human plasma can be abolished or minimized by means of human plasma injections, if the treatment is continued long enough.

Discussion.

The present experiments indicate that human plasma causes, in the majority of cases, a decrease in the number of red cells of rabbit blood in vitro as well as in vivo, *i. e.* the occurrence of natural anti-rabbit haemolysins in human blood is not unusual, and should therefore be considered when investigating the haemopoietic activity of human plasma by injecting it into rabbits. Haemolysis in vivo is, however, stronger and more constant than haemolysis in vitro, and thus the haemolytic effect of human plasma injection can be evaluated by a test in vitro only with moderate certainty.

It is suggested that incubation with rabbit blood cells diminishes the activity of human plasma to haemolyse rabbit blood. On the basis of the present data it cannot be decided, whether incubation with rabbit blood cells might be of practical value for abolishing the haemolytic activity of human plasma in haemopoietic research; this is even more questionable as the effect of incubation on the haemopoietic principles is not known.

According to the present data, repeated injections of small amounts of human plasma decrease the susceptibility of rabbit blood to undergo haemolysis in the presence of human plasma in vivo and in vitro; if a sufficient number of injections at suitable intervals are given, full immunity against human plasma may be reached. This is in conformity with the observation of BONSDORFF and JALAVISTO that rabbits immunized by means of repeated injections of human plasma respond to haemopoietically active human plasma with an increase of red blood cells, while in untreated rabbits the red cell count remains at the initial level or decreases.

As the safest way to control the rabbit red cell haemolysing effect of human plasma in experiments on haemopoietic sub-

stances immunization by repeated injections of human plasma is suggested.

There is no indication that the natural blood group of the human donor were of any importance for the rabbit red cell haemolysing effect, nor for the immunization of rabbits against human plasma.

Summary.

The rabbit red cell haemolysing property of human plasma was investigated in vivo and in vitro. Human plasma caused, in most cases, a decrease in the red cell count of rabbit blood in vitro as well as in vivo, though the reaction was stronger in vivo. Repeated injections of human plasma into rabbits decreased the susceptibility for haemolysis in vivo and in vitro. Incubation with rabbit cells diminished the activity of human plasma to haemolyse rabbit cells in vitro.

References.

Bonsdorff, E., and E. Jalavisto, Acta Physicol. Scand. 1948. *16.* 150.

Castle, W. B., T. H. Ham, and S. C. Shen, Trans. Ass. Amer. Phys. 1950. *63.* 161.

Davidsohn, I., and K. Stern, Cancer Res. 1949. *9.* 426; 1950. *10.* 571.

Ehrlich, P., and J. Morgenroth, Berl. Klin. Wschr. 1899. *36.* 481.

Ersley, A., Blood 1953. *8.* 349.

Loeb, L., A. Strickler, and L. Tuttle, Virchows Arch. 1910. *201.* 5.

Sievers, O., Acta Path. Microbiol. Scand. 1937. *14.* 553.

Stuart, C. A., J. Tallman, and E. Anderson, J. Immunol. 1935. *28.* 75.

Wasastjerna, C., Acta Med. Scand. 1948. *32.* 32.

— Ibidem 1951. *140.* Suppl. 258.

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The Effect of Thyreoid Hormone on the Mechanogram of the Isolated Rat Auricle.

By

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The clinical observation of heart failure and muscular weakness in patients with thyreotoxicosis have probably stimulated the many experimental studies concerned with the action of thyreoid hormone on the heart. The main question has mostly been whether the thyreoid hormone acts directly upon the heart muscle or indirectly by way of the autonomous nervous system. The object of study par préférence has been the isolated heart of different animals.

LEWIS and MC EACHERN (1931) working on isolated rabbit hearts and auricles noted that the frequency of beat in hearts isolated from thyreotoxic animals was much accelerated when compared with that recorded with hearts from normal animals. MC EACHERN (1932) was, furthermore, able to show that the oxygen consumption of the isolated beating auricles was higher in those isolated from thyreotoxic guinea-pigs than in auricles from normal animals. FERRANINI (1936) confirmed the results concerning increased rate of action by using strips of isolated auricles from guinea-pigs treated with thyroxin and thyreotropic hormone. The effect of thyroxin was greater after 3 than after 8 days of thyroxin medication. DEMOOR (1936) produced by administration of thyroxin during 5—10 days highly toxic effects *i. e.* irregularity of the beat with disturbances of conduction and initiation of the impulses in the isolated auricle of the rabbit. Whereas the peripheral effects of thyreoid hormone manifested in acceleration of the heart rate and irregularity in the initiation

Table

Temperature		27°		
Initial distension (mm)		0	3.15	6.30
Frequency (per minute)	Control	130 ± 8	120 ± 8	123 ± 9
	Thyranon	166 ± 9	166 ± 9	159 ± 11
	Difference	36 ± 12	46 ± 12	36 ± 14
Amplitude (arbitrary units)	Control	2.5 ± 0.37	5.6 ± 0.57	6.5 ± 0.64
	Thyranon	1.7 ± 0.18	4.0 ± 0.46	5.3 ± 0.50
	Difference	0.8 ± 0.41	1.6 ± 0.73	0.8 ± 0.81
Duration of contraction (1/100 sec)	Control	10.8 ± 0.32	12.2 ± 0.25	13.1 ± 0.25
	Thyranon	9.4 ± 0.26	10.7 ± 0.34	11.8 ± 0.33
	Difference	1.4 ± 0.41	1.5 ± 0.42	1.3 ± 0.41

and conduction of impulses in the auricle, are well established, it seems unsettled whether it has any effect on the mechanical properties of the auricular muscle contraction. It is, however, possible to study the effect of thyreoid hormone on the heart by analysing the isometric mechanograms of the auricular contractions at various rates of action. The frequency of the spontaneously beating auricle can *viz.* easily by altered by changes in temperature. The right auricle of the rat seems well suited for such studies.

Methods.

The series consisted of 7 normal control rats weighing 195 g on an average and 7 experimental rats (average weight 198 g) which were injected subcutaneously with the thyreoid preparation "Thyranon" (Organon) once a day on three successive days. The dosis was 0.4—0.5 ml the content of iodine of the preparation being 0.1 mg/ml. The experiments were performed on the following day after the last injection. The isolation of the auricle was performed as follows. The rats were anaesthetized with Nembutal, the anterior part of the thorax was removed and a tie was placed in the cranial v. cava 7 mm above its auricular junction. A second auricular tie was fastened between the right auricle and ventricle. By lead of the ties the auricle was cut away with scissors. It was placed in an isolated organ bath with 50 ml LOCKE's solution the composition of which was as follows: NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5, glucose 1.0 and H_2O ad 1,000.0. A continuous O_2 stream was lead through a fairly fine glass sinter.

1.

32°			37°		
0	3.15	6.30	0	3.15	6.30
220 ± 10 268 ± 14	221 ± 9 271 ± 10	218 ± 8 267 ± 10	324 ± 7 412 ± 23	320 ± 8 412 ± 19	320 ± 9 405 ± 18
48 ± 17	50 ± 13	49 ± 13	88 ± 24	92 ± 20	85 ± 20
1.7 ± 0.22 1.3 ± 0.21	5.1 ± 0.72 3.2 ± 0.56	6.8 ± 0.52 4.2 ± 0.61	1.4 ± 0.27 0.6 ± 0.12	3.4 ± 0.61 1.7 ± 0.38	4.9 ± 0.48 2.9 ± 0.55
0.4 ± 0.30	1.9 ± 0.91	2.6 ± 0.80	0.8 ± 0.30	1.7 ± 0.72	2.0 ± 0.73
7.1 ± 0.14 6.7 ± 0.21	8.3 ± 0.24 7.5 ± 0.24	9.3 ± 0.24 8.1 ± 0.24	5.3 ± 0.17 4.8 ± 0.08	6.1 ± 0.14 5.4 ± 0.16	6.4 ± 0.25 6.1 ± 0.12
0.3 ± 0.25	0.8 ± 0.34	1.2 ± 0.34	0.5 ± 0.19	0.7 ± 0.21	0.3 ± 0.28

The orienting experiments were made with isotonic lever on smoked paper, but for the final experiments a piezoelectric crystal recording system constructed from a "Ronette" (trade mark) pick up set by substituting the needle by a 22 mm long stealen hook was used. The upper fastening tie of the preparation could easily be changed over from the recording isotonic lever into the hook. The load corresponding to the resting level of the lever (marked in the graphs with 0) was 140 mg. The crystal and the hook was fastened to a micrometer stand and could be raised and lowered by turning the micrometer. The tension of the auricle could thereby be regulated. The mechanograms were registered with a Triplex electrocardiogram (IVth connection) through the condenser-resistance coupling unit belonging to the pulse registering device of the electrocardiograph. The registered amplitude is proportional to the tension developed. The properties of the recording system are presented in detail elsewhere (HIRVONEN 1954). In figure 1 a mechanogram thus registered is seen.

The observations were made at three different temperatures: 27°, 32° and 37° C. In all preparations two series of observations were made at each of the temperatures. In order to avoid effects of fatigue the

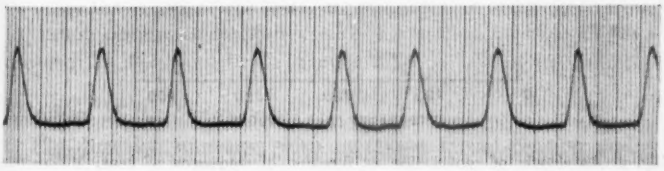

Fig. 1. Mechanograms recorded with piezoelectric myograph from isolated right, spontaneously beating rat auricle.

sequence of observations was alternated so that at every temperature the preparations were on an average of equal "age" and equally fatigued. The curves to be represented show the averages. The means and the differences of means between control and experimental values with their mean errors are shown in table 1.

Results.

Frequency of beat.

The frequencies of the isolated auricles from the thyranon rats are on an average 27 per cent higher than those of the auricles of normal animals. The percentage is not dependent upon temperature *i. e.* in 27° C it is 32, in 32° 23 and in 37° C 28 per cent. When the temperature coefficient $Q_{10°}$ is calculated it is found to be practically the same both in normal and in thyranon auricles $Q_{10°} = 2.7$ (controls) $= 2.5$ (thyranon). The initial tension does not affect the frequency.

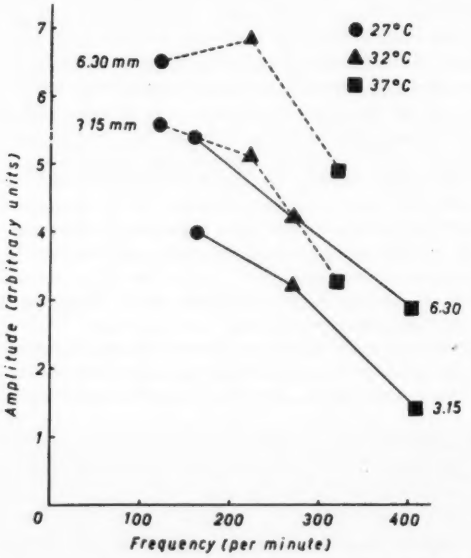

Fig. 2. Amplitude of contraction as function of frequency of beat. Millimeters marked on the curves indicate initial distension. Thyranon treated rats —, control rats — — — — —.

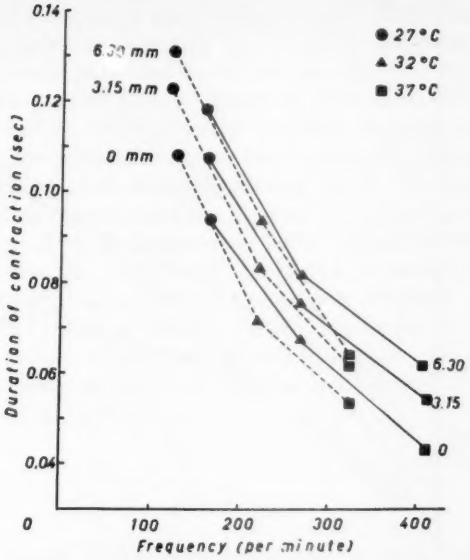

Fig. 3. Duration of contraction plotted against frequency of beat. Millimeters indicate initial distension. Designations as in fig. 2.

Amplitude (force) of contraction.

With increasing initial tension the amplitude of contraction is seen to increase nearly linearly both in normal and thyranon treated preparations. The temperature between 27° or 32° C does not affect the amplitude of normal preparations but decreases it clearly at 37° C. In the thyranon treated rat preparations the amplitude of contraction is more affected by differences in temperature and, furthermore, it is with all tensions and at each temperature considerably smaller than that of the control preparations. If the amplitude of contraction is plotted against frequency of beat, it may be noted that the amplitude is more decreased in thyranon preparations than would be expected on basis of the increased frequency of beat. (Figure 2.)

Duration of the contraction.

The duration increases with increased initial tension and decreases with increasing temperature. It is clearly shorter in the thyranon treated rat auricles but as seen from figure 3 this pheno-

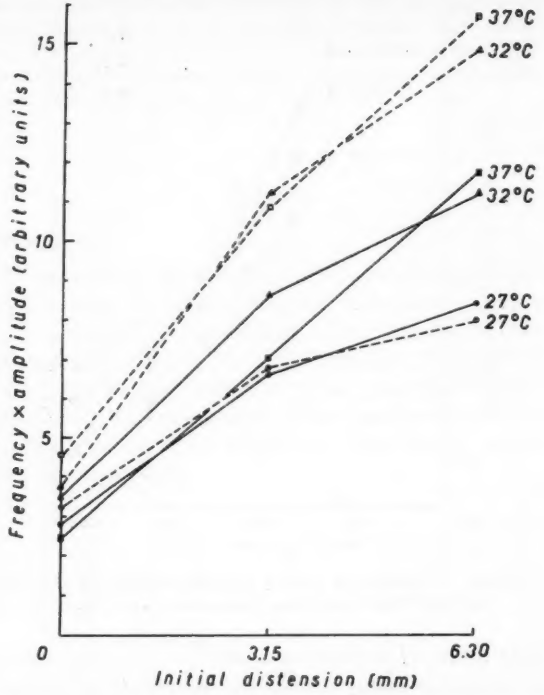

Fig. 4. Frequency of heat × amplitude of contraction with different initial distensions. Designations as in fig. 2.

menon can be fully explained by the difference in frequency of beat. The control and thyranon values lie practically on the same lines. The temperature coefficient of duration Q_{10° is 2.0 (control) and 1.98 (thyranon) *i. e.* the same in both cases. It may be pointed out that the Q_{10} values for frequency and for duration seem thus to differ and may be regarded as an indication of the fact that frequency and duration of beat are in spite of interdepence differently affected by alterations in temperature.

Mechanical efficiency of the auricle.

Since the registering has been isometric the working capacity of the auricular muscle cannot be calculated. As a measure of its mechanical functioning capacity the product amplitude *i. e.* force of

contraction × frequency may be used instead. This measure would represent a magnitude analogous to cardiac output, if the force of contraction is paralleled to the volume output of the heart. In figure 4 the product force × frequency is given in different temperatures and at different initial tensions. As may be seen the product is the same both at the temperature 32° and 37° in the control auricles, but definitely smaller at 27° C. This may be interpreted as showing that the increase in contraction force with the lowering of temperature compensates the fall in frequency at 32° C but not any more in 27° C. In the thyranon treated rat auricles the product of force × frequency is definitely smaller at 32° and 37° C, than in controls. In 27° C, however, no difference is seen between controls and thyranon auricles. Obviously the higher frequency of thyranon auricles compensates for the decreased force of contraction.

Discussion.

The peripheral action of thyroxin and thyreoid hormone on metabolic processes seems to be well established. Thus, as mentioned in the introduction the oxygen consumption is increased in organs isolated from thyreotoxic animals, *e. g.* in isolated auricles of rodents (Lewis and Mc Eachern 1931, Mc Eachern and Andrus 1931, Mc Eachern 1932).

In the case of spontaneously beating hearts or auricles the increased uptake of oxygen may be explained by the rapid rate of heart action and the hypertrophy of the heart muscle (Dock and Lewis 1932). Similarly, when it is found that auricles from thyreotoxic animals are more sensitive against deprivation of oxygen (Andrus and Mc Eachern 1932) and that the isolated heart uses more sugar (Ambrus 1929) it could be explained on basis of the greater demand for oxygen and nutrients in the thyreotoxic organs. According to Kommerell (1931) no difference in the mechanical efficiency of the muscle of normal subjects and of patients with Graves' disease can be seen in a careful study of respiratory metabolism under exercise. The biological energetic process would, according to him, not be altered in thyreotoxic patients.

In the experiments presented in this paper it was, however, shown that not only the frequency of beat but also the contrac-

tion force of the isolated rat auricle was affected by the thyreotoxic state of the animal. The reduction in contraction force was greater than could be accounted for by the increased frequency of beat. Thus if a thyreotoxic auricle has to maintain a certain mechanical performance level per unit of time, it has to beat at a greater frequency than a normal auricle. If this observation could be generalized and one would suppose it to be valid for the whole heart muscle, it would make the disturbances in heart action and the frequent heart failures in Graves' disease more easily understood.

Summary.

1. The effect of short-term (3 days) treatment with thyreoid hormone ("Thyranon", Organon) on the isolated spontaneously beating right auricle of rat, was investigated.

2. The auricular contractions were registered isometrically with a piezoelectric crystal myograph and a Triplex electro-cardiograph.

3. The effect of temperature and of initial tension, *i. e.* fibre length on the mechanograms of auricles from normal and thyreotoxic animals, was studied.

4. It was found that the effect of thyranon consisted in increased frequency and decreased contraction force of the auricle. The reduction in the contraction force was greater than could be accounted for by the increase in frequency. The diminution of contraction force leads to a decrease in the product of force and frequency.

References.

Ambrus, G., Biochem. Z. 1929. *205*. 194.
Andrus, E. C. and D. Mc Eachern, Quot. by Mc Eachern.
Demoor, P., C. R. Soc. biol. Paris. 1936. *123*. 536.
Dock, W. and J. K. Lewis, J. Physiol., London 1932. *74*. 401.
Ferranini, A., Arch. int. Pharmacodyn. 1936. *54*. 299.
Hirvonen, L., unpublished.
Kommerell, B., Deutsch. Arch. Klin. Med. 1931. *171*. 308.
Lewis, J. K. and D. Mc Eachern, Bull. Johns Hopkins Hosp. 1931. *48*. 228.
Mc Eachern, D., Ibidem 1932. *50*. 287.
— and E. C. Andrus, J. Clin. Invest. 1931. *10*. 653.

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L'influence de la fréquence de changement de l'intensité de la sensation sur la durée de la sensation.

Par

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Dans la formation de la notion du temps, la sensation de la succession et de la durée ont une signification décisive. (BERGSON 1913, KASTENHOLZ 1922). La sensation de la durée se base sur l'apprehension qu'un contenu de sensation qui existait juste (ici et maintenant), existe aussi sur le moment (voir: REENPÄÄ 1947). L'expérience de la succession se base sur le changement, un contenu de sensation qui existait juste (ici et maintenant) n'existe plus maintenant. Nous remarquons de ces définitions, que la durée et la succession sont des phénomènes corrélatives, qui dépendent l'un de l'autre.

Le changement, l'événement est donc la base du temps, mais c'est aussi le caractère de la vie: «Sein ist Zeit» (HEIDEGGER 1929). La signification de l'événement pour la notion du temps est prouvée entre autres par cette expérience connue: qu'une journée pleine d'événements donne l'impression d'être plus longue — dans la rétrospective — qu'une journée pauvre d'événements. (STRAUS 1947). Expérimentalement on a pu prouver cet événement entre autres par l'influence des changements de l'espace: plus grande est la vitesse de l'objet vu, plus grand le temps est surestimé (ROELOFS et ZEEMAN 1951). Dans les recherches sur le rythme on a trouvé que les rythmes lents sont surestimés, tandis que les rythmes rapides sont sousestimés quand ils sont reproduits. HARRELL (1937) avait comme rythme de fréquence indifférente 2—3/sec. Les événements ont donc une vitesse préférée,

vers laquelle l'événement plus rapide aussi bien que l'événement plus lent sont dirigés par une tendance dans notre observation.

Sur cette base on peut, selon mon opinion, expliquer l'expérimentation suivante de HARRELL. Il y a, comme on sait, dans l'homme une tendance de diviser les rythmes égaux en battements de 2, 3 ou plus souvent en 4. HARRELL a pu prouver que cette formation spontanée d'unités est une fonction rythmique, telle que la division se fait en unités qui deviennent de plus en plus grandes avec l'accroissement de la rapidité. La formation du rythme préféré se fait le plus souvent dans le battement de 4, tel que les rythmes les plus rapides sont formés dans des unités plus larges, tandis que les rythmes lents sont mis dans des unités plus condensés. La formation d'unités donne l'impression que le rythme devient plus lent qu'il est objectivement, ainsi par exemple, selon cette explication on peut s'imaginer — au cas du rythme préféré, que les battements en 4 se forment en unités qui a leur tour forment un rythme égal. En changeant les groupements selon la vitesse du rythme, l'homme montre une tendance de conserver la fréquence d'événement près du préféré. Cette pensée est aussi confirmée par l'expérience contraire de HARRELL: qu'en changeant exprès de groupement, aussi le rythme préféré change, et cela se fait plus rapidement si les unités sont faites plus grandes.

Méthode.

Des recherches sur la signification du nombre des événements sur la notion du temps ont été faites, expérimentant avec 20 étudiants de l'Université dans l'age de 19 à 22 ans. Chacun d'eux était prié de fixer une durée de temps de 10 secondes sur la manière suivante: Après avoir entendu le signal au commencement, ils donnaient eux-mêmes le signal pour terminer les 10 secondes. Dans la mesure où le chronomètre montrait une différence considérable des 10 secondes demandées, on cherchait une durée de temps à laquelle la personne en question donnait une durée, laquelle — mesurée sur le chronomètre — arrivait au plus près de 10 secondes.

Après cela on appliquait à l'étudiant un son d'une vibration de 1,000 Hertz, et il donnait le signal au moment où il pensait avoir entendu le son pendant 10 sec. Pendant l'expériment on changeait l'intensité du son pendant des intervalles réguliers; utilisant uniquement les intensités de 55 et 75 décibels, toujours commençant avec l'intensité de 55, tellement que dans le premier expériment de la serie le changement d'intensité était fait toutes les 4 secondes, dans le second expériment toutes les 2 secondes, après toutes les 1, 1/2, 1/4 et 1/8 secondes. Le projet de cette série se basait sur des directions, données par les ex-

périmentations préliminaires. Par des expérimentations déjà faites par WOODROW, (1951) on sait que, si les séries d'expérimentation pour deviner la durée du temps sont répétées trop souvent avec la même personne, elles changent les résultats. Ces résultats montreront toujours l'influence de la série d'expérimentation sur les expériments individuels. Pour éviter ceci on donnait à la même personne seulement une série, et elle était exécutée selon une fréquence croissante du changement d'intensité.

Résultats.

Les résultats qui se trouvent ci-dessous sont les moyens de plusieurs personnes. On les obtenait sur la manière suivante: on prenait comme valeur fondamentale les secondes de la première expérimentation du son et l'indiquait avec 100. Le résultat de chaque expériment suivant était conté en pourcentage de la première valeur fondamentale. Nous remarquons que dans la mesure où la fréquence de changement est augmentée, le temps est surestimé, Ce phénomène atteint son maximum quand la fréquence de changement est 2/sec. Après cela une réduction dans la surestimation du temps commence de nouveau. Comme on voit ci-dessous, la surestimation est d'une compétance statistique depuis la fréquence de changement de 1/sec. La diminution de la surestimation entre la fréquence de changement de 2/sec. et le moment de 8/sec. est aussi d'une compétence statistique ($\varepsilon(M_2 - M_8) = 3.9$).

Fréquence de changement, changement/sec:	1/4	1/2	1	2	4	8
M	100	97	86	80	85	92
ε		1.1	2.5	2.7	2.3	1.9

La dépendance de la duration des sensations de la fréquence du changement de l'intensité des sensations. M = valeur moyenne. ε = erreur de standard.

Discussion.

Le maximum de surestimation se dirige vers le point de fréquence du groupe de la même grandeur, où HARRELL prouvait dans ses recherches sur le rythme être le rythme d'indifférence. Dans les recherches sur la duration on a obtenu comme intervalle d'indifférence surtout 0.8—0.6 secondes (voir WOODROW 1951). Il est tout à fait selon les expectations que l'accélération

de la vitesse des changements est la cause de la surestimation du temps. (Voir aussi par exemple PHILIP 1947.) Si la vitesse des changements surpasse le rythme d'indifférence on voit quand-même une diminution de la surestimation. Je présume que l'explication de ce phénomène est celle, que les unités d'événements se suivent si rapidement les uns les autres que la perception exacte comme unités indépendantes est devenu difficile et le rythme commence à donner de plus en plus l'impression d'une sensation homogène; l'impression du changement diminue. L'expériment prouve donc, que l'augmentation de la fréquence de changement des sensations, examiné avec l'aide de changement d'intensité, a comme conséquence une surestimation de la durée, laquelle quand-même diminue si la fréquence de changement surpasse la limite du rythme d'indifférence. Sur la base de cet événement je propose la supposition: que la capacité d'apercevoir les changements devient graduellement plus difficile si la fréquence des changements surpasse la limite du rythme d'indifférence.

Résumé.

Dans l'article ci-dessus la signification de la fréquence du changement des sensations pour la notion du temps est examinée. Les expériences montrent, que l'accroissement de la fréquence du changement de l'intensité de la sensation d'un son de 1,000 Hertz, est la cause d'une surestimation de la durée, laquelle de nouveau diminue si la fréquence du changement surpasse la limite du rythme d'indifférence. La cause de ce phénomène est examinée.

Litterature.

BERGSON, H., Creative Evolution, New York 1913.
HARRELL, T. W., J. gen. Psychol. 1937. *17.*
HEIDEGGER, M., Jb. philos. phaenomenolog. Forsch. VIII, 2 Abt.
KASTENHOLZ, J., Arch. ges. Psychol. 1922. *43.*
PHILIP, B. R., J. gen. Psychol. 1947. *36.*
REENPÄÄ, Y., Über Wahrnehmen, Denken und Messendes Versuchen. Leiden 1947.
ROELOFS, C. O. et W. P. C. ZEEMAN, Acta Psychol. Hague 1951. *VIII.* 2.
STRAUSS, E. W., S. med. J. 1947. *40.*
WOODROW, H., Handbook of Experimental Psychology, New York 1951.

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Patterns in Perceptual Constancy Experiments and the Phantom Limb Phenomenon in Amputees.

By

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The phenomena of "constancy" in perception (phenomenal regression to the "real" object according to THOULESS) has from the time of HELMHOLZ and HERING occupied a central position in the theories of perception. It is the purpose of this paper to tackle this problem from a new, perhaps, strange-looking point of view.

The phantom phenomenon in amputees is the most striking illustration of the existence of a "body image". This again, as representing a relatively constant percept of our own in many ways changing body with its variable states of nervous stimulation, may be ranged among other thing constancy phenomena. The "body image" could be called "body shape constancy" in analogy to *e. g.* visual "shape" and "size" constancy. The "real" body would then correspond to the "real" size or shape of an object. "Real" means here simply a conceptual, common sense "physical" characteristic of the object.

It is perhaps necessary to point out that the phantom limb persists only if some peripheral irritation exists, but that its phenomenology and structure is mainly governed by the functional organization of the sensory and motor cortex (JALAVISTO 1947, JALAVISTO and SOURANDER 1948). The phenomenology of the phantom limb discloses traits, which are dependent upon

purely conceptual variables of the perceptual situation. Thus *e. g.* the mere approaching of an obstacle may induce alterations in the position of the phantom arm or its temporary disappearance. (This experiment is called "obstacle shunning experiment" and has been described in a previous paper, JALAVISTO 1950.)

The existence of "thing constancy" or phenomenal regression toward the "real" object is dependent upon a) the recognition of cues which characterize the perceptual situation (THOULESS 1932) and b) the adaption of the percept to this situation. The same holds true for the phenomenon of obstacle shunning of the phantom in amputees. It is, therefore, not without interest to examine the regression to the "real" object in *e. g.* visual perception together with observations on the "obstacle shunning" of the phantom limb.

Starting from this reasoning various experiments adapted to disclose in some form the existence of perceptual constancy, were performed in amputees. The experiments were selected as to represent different sensory modalities.

The obtained data were examined in view of whether a typical pattern in the phantom limb phenomenon was correlated to some typical behaviour in the perceptual constancy experiments.

Methods and Material.

The experiments were performed in 159 upper limb amputees. All tests could not, unfortunately, be carried out on every patient. The series (with 19 additional cases in which no experiments on constancy were performed) has been presented in a previous paper which was mainly concerned with the somatic aspects of the phantom limb phenomenon (JALAVISTO and SOURANDER 1948).

The following tests and observations were selected for treatment in this paper.

1) *Observations of the behaviour of the phantom* when the stump is placed near a wall so that the phantom would occupy a place within the wall if perceived in its usual position ("Obstacle shunning experiment").

2) *Subjective weight of the phantom arm.* Equality of weight of the intact arm and the phantom arm may be considered as an indication of "proprioceptive arm weight constancy" (JALAVISTO 1946).

3) *Size-weight illusion* which according to THOULESS may be ranged among the phenomena of regression toward the "real" object — *i. e.* toward constancy of specific gravity.

4) Matching color-wheel grays in different illuminations in order to disclose *constancy of brightness or albedo* (BRUNSWIK 1934).

5) Arm flexion experiments in which the patient had to reproduce arm movements of equal amplitude. Measured equality of the phenomenal amplitude of successive flexions may be termed "*proprioceptive movement amplitude constancy*" (JALAVISTO, LEPPÄNEN et al. 1937).

1) *Obstacle shunning experiment.*

Particular attention was given to the following points:

a) Was the phantom present at the beginning of the examination?

b) Was the phantom limb perceived as being within the stump or outside it?

c) Did the phantom sensation disappear or otherwise shun the obstacle or did it become located within the stump when the arm stump was placed near a wall or other obstacle.

The patients were then ranged in the following groups:

1. Phantom was not present at examination (n = 20; 12.5 %).
2. The phantom was located within the stump (n = 20; 12.5 %).
3. The phantom "shuns" an obstacle (disappears, bends to side or moves within the stump) (n = 33; 21 %).
4. The phantom occupies a place within the wall, *i. e.* is not affected by the situation in the obstacle shunning experiment (n = 59; 38 %).
5. Statements are vague, no clear "shunning" (n = 25; 16 %).

2) *Subjective weight of the phantom.*

When the patients were asked to report on the sensation of weight of their phantom arm the judgements were classified as follows:

a) The phantom arm is lighter than the intact arm. This behaviour corresponds to the actual "stimulus" situation — the lost weight of the limb influences the phantom sensation. This perceptual pattern is thought to correspond to lacking or slight regression to the "real" object. Designation — C.

b) The phantom arm might occasionally be lighter but as a rule there is no difference of weight, or the phantom is even heavier than the healthy arm. Designation $\pm$ C.

c) The phantom arm and the healthy arm are phenomenally of equal weight. This perceptual pattern may be paralleled to experiments where the regression to the object character is of high degree. The phantom arm has the same weight as a "real" arm in spite of the fact that the stump is considerably lighter than the intact arm. Designation + C.

3) *The size-weight illusion.*

The patients were asked to sort a series of different weights and to indicate which of the weights was equal to a smaller standard weight. The series of weights consisted of wooden hollow eggs (children's toys) the standard weighing 75 g, its volume being 28 ccm. The volume of the bigger eggs was 135 ccm and their weights varied as follows: 75, 90, 100, 105, 120, 135, 150, 165 and 175 gms. The weights of 100 and 105 gms could as a rule not be discriminated but all other weights could by most subjects be put in the right sequence. Those experiments in which the sorting was not correct were discarded. The

remaining might be considered as being performed by people with fairly similar discriminatory ability in weight experiments. The size-illusion was present with striking intensity in all subjects. The test was performed in 108 subjects but 25 failed in the sorting of the weights. The number of failures was relatively greatest in those with the apparently biggest illusion. The distribution of values is abnormal there being an excess toward values indicating big illusion. As will be shown later this abnormality in distribution can hardly be explained by the smallness of the number of observations, it seems to be inherent to the phenomenon in question.

4) *Constancy of brightness or albedo.*

If interindividual differences in perceptual behaviour are to be examined the experimental conditions must be chosen such that the dispersion of performance values are great. In many experiments this principle has not sufficiently been taken into account. By the following arrangement it was possible to meet this requirement.

The patient stood on 2.75 meters distance from a color mixing wheel, which could be regulated without interrupting the rotation. The color-wheel was illuminated by a lamp at the same distance from the color-wheel as the patient. In an other room with black walls and black furniture was a second color-wheel in much dimmer illumination. The ratio of the illuminations was 55 : 1. The wheel was at a distance of 7.0 meters from the subject. The standard stimulus consisted of a sector of 90° black and 270° white. The patient looked through an opened door to the darkroom. Both rotating discs were simultaneously visible and the patient was asked to report when they looked being of same brightness. The experimenter turned slowly the variable disc starting alternatively from black or white until the patient indicated equality of hue. The experiment was repeated 6 times (5 in the beginning) and the average value for the white sector was taken as the equivalence value (R).

Photometric determinations of the brightness of both rotating discs were carried out and calibration curves of the variable wheel were constructed.

This is shown in figure 1, in which the photometer reading is plotted against degrees of white sector. Since the illumination was somewhat variable dependent upon fluctuations in the mains voltage, the calibration did not always yield the same values. Two extreme cases are shown in the figure 1.

In order to estimate the degree of "constancy" the ratio $\frac{R-S}{A-S}$ (Brunswik ratio = BR, Brunswik 1934) was calculated from the calibration curves, S being the "stimulus" value *i. e.* a standard mixture with 270° white (0.32—0.35 lux) A representing the "object"-value *i. e.* the photometer reading corresponding to the same 270° white in the variable wheel but in the stronger illumination. R represents the value in lux corresponding to the found white sector degree representing equivalence of subjective brightness and was read from the calibration curve. It was shown that the values of the Brunswik

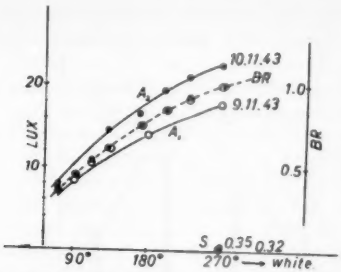

Fig. 1. Calibrations of the color mixing wheel and the corresponding BR = $\frac{R-S}{A-S}$ values. Abscissa: Degrees of white sector. Ordinate: Photometer readings in lux.

ratio did practically not differ in spite of differences in the calibration curves (fig. 1 black and white circles). In figure 2 A the distribution of the equivalence (R) values are shown. As may be seen values of Brunswik ratio below 0.50 are rare, whereas an "overconstancy" (Brunswik ratio > 1.00) was found in 16 out of 135 cases. Most values lie between the ratios 0.75 and 1.00. The distribution is not quite normal but the excesses in both ends of the curve are not great. The distribution of the mean errors of the means are shown in figure 2 B.

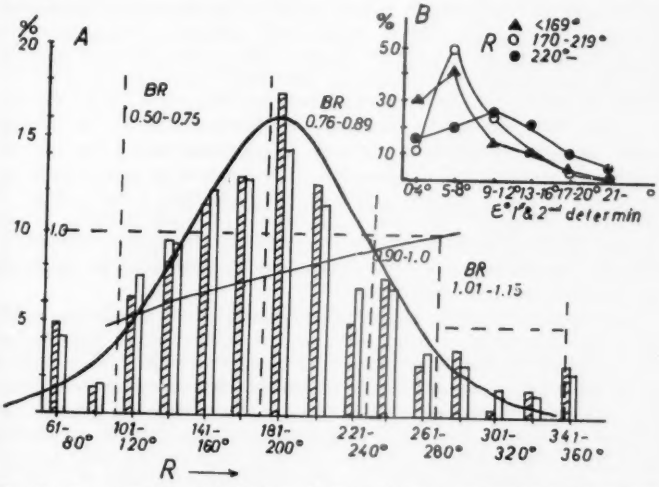

Fig. 2. A, percentile distribution of the subjective equivalence, R, values. Hatched columns 1st determinations only. White columns 1st and 2nd determinations. B, percentile distribution of the mean errors of the means, ε, in the different ranges of R.

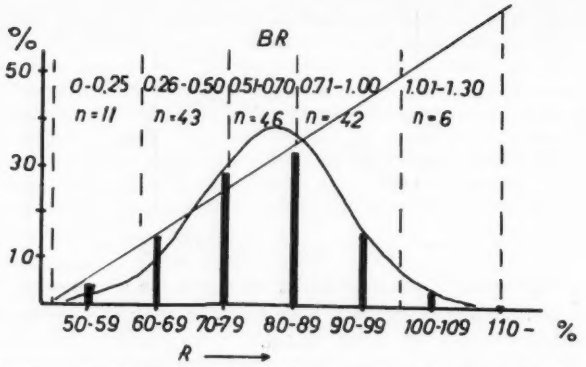

Fig. 3. Percentile distribution of the values corresponding to arm flexion amplitude equivalence, R, and the corresponding BR values.

5) *Experiments on constancy of amplitude of active arm movements.*

If one has to reproduce by flexion of the arm movements of equal amplitude there is a tendency to overestimate those starting from a more flexed position of the arm. It has been shown that this overestimation *i. e.* shortening of movements with progressive flexion is very regular especially in children and those blind from birth. This overestimation can be shown to correspond to the anatomical shortening of muscles during the flexion equality of sensation corresponding to equal shortening of the flexor muscles (or in extension movements to equal shortening of the extensor muscles) (JALAVISTO et al. 1937).

The shortening of the flexor muscles when starting from 90° flexion is about 3/2 times greater per grade of flexion than the shortening in the extended position of the arm. Overestimation due to "muscle shortening equivalence" is, however, not constant in adults and the successive arm movements judged as being of equal length are often practically of same "objective" amplitude. In these subjects it can be postulated that there exists a "constancy of subjective amplitude of active movements" and its degree can be calculated by applying the principle of Brunswik ratio.

The experiments were performed with help of an apparatus, which consisted of a rotating metal arm, and a friction-wheel with strings for recording of the movements. The strings were lead over pulleys to an inkwriter on the surface of a kymograph not visible to the subject. On the axle of the rotating bar an elbow rest was placed. The arm of the subject was fixed to the bar with the help of a celluloid glove upbolstered with cotton. The movements of the bar were damped by pressing a friction pad against the wheel with the help of a string.

The arm of the subject was placed in the apparatus and he was asked to flex his arm starting from the extended position of the arm (flexion angle 10°) and then to try to reproduce successive flexions

all of the same amplitude until his arm was fully flexed. Visual control was eliminated with black spectacles. The experiment was repeated 10 times with each patient. The result was calculated as follows. The summated length of the first half of the flexions performed in one experiment was taken as reference unit and the summated amplitude of the rest of the flexions was expressed as percent of the reference unit. The average of the percentage values of ten curves was the final rating. The value 50 % was considered as the S "stimulus"-value of the Brunswik ratio since it approximates the muscle shortening equivalence determined from measurements made on human skeletons. (JALAVISTO, LEPPÄNEN et al.), 100 % representing the "object"-value A. Figure 3 shows the distribution of the equivalence (R) values and their place in the Brunswik ratio scale. As seen from the figure there are only few cases of "overconstancy". The distribution is normal.

Results.

Intercorrelation of the various constancy tests.

If the results obtained in the same subject with different tests are compared, no significant correlation can be found. Thus those exhibiting great "constancy" of brightness may react in any possible way in *e. g.* the arm movement amplitude experiment, the phantom may either show a "constancy" of arm weight or not, and the size-weight illusion may be great or small. Obviously the tests mostly measure the behaviour of the subject in that particular test. The following table 1 will indicate this lack of correlation between the tests. It is obviously not necessary to calculate the correlation coefficients. The conclusion seems justified that the tests do not reveal any general intermodal tendency inherent to the subject either toward thing constancy behaviour or to its opposite. However, the interindividual differences are so great that the question cannot be left at that.

Phenomenology of the phantom limb as a differentiating principle.

The behaviour of the phantom limb in the obstacle shunning experiment has so far not been considered. In a previous paper it was shown that the phenomena of "obstacle shunning" and the disappearance of the phantom were more common in the amputees less than 25 years old. It was postulated that the behaviour of cases belonging to the groups 1—3 (see methods, p. 169) represents an adaptation phenomenon whereas the groups 4 and 5 (see methods p. 169) with their rigid, fixed phantoms are

Table 1.

Constancy of brightness \ Arm movement amplitude constancy	BR 0—0.50 (53—75 %)	BR 0.51—0.70 (76—85 %)	BR > 0.70 (≧86 %)	
BR < 0.75 (65°—169°)	14	17	18	49
BR 0.76—0.89 (170°—219°) ..	15	16	17	48
BR 0.90— (220°— ..	17	8	8	33
	46	41	43	*130*
Constancy of brightness \ Arm weight constancy	**— C**	**± C**	**+ C**	
BR < 0.75 (65°—169°)	19	15	12	46
BR 0.76—0.89 (170°—219°) ..	15	16	14	45
BR 0.90— (220°— ..	8	8	15	31
	42	39	41	*122*
Arm movement amplitude constancy \ Arm weight constancy	**— C**	**± C**	**+ C**	
BR 0—0.50 (53—75 %)	26	10	15	51
BR 0.51—0.70 (76—85 %)....	14	15	14	43
Br > 0.70 (≧86 %).....	16	14	15	45
	56	39	44	*139*

more resistent to adaptative motivations (JALAVISTO 1950). If the representation of these groups in the different constancy (BR) ranges of the experiments are examined a quite curious

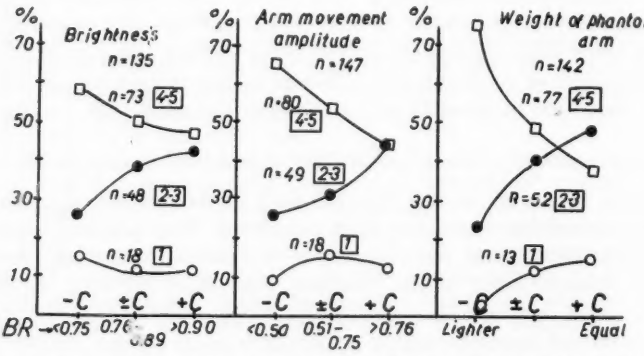

Fig. 4. Percentage of absent (1) "adaptable" (2—3) and "rigid" (4—5) phantoms in the — C, ± C and + C ranges of the different constancy experiments.

fact may be seen. The relative number of those cases belonging to the group with "adaptable" phantoms (groups 2—3) is regularly greatest among those subjects with the highest BR values (or other + C groups) and least in the — C groups. The class limits may be altered without materially altering the results. Figure 4, which is constructed so as to show the percentage of cases with "adaptable" phantoms (groups 1, 2—3), and "rigid" (groups 4—5) in the — C, ± C, and + C groups shows a very regular decrease in the percentage of "rigid" phantoms and an increase of "adaptable" phantoms with increasing constancy.

However, the decreases and increases are not great; as a matter of fact it is highly significant only in the phantom weight observations and probably significant in the arm movement amplitude experiments but not significant in the brightness experiments (Table 2). Without making any statement of the meaning of this finding it is thought that, anyhow, it calls for closer examination of the constancy behaviour *within* a group of cases differentiated from the whole by the "adaptability" of the phantom.

Table 2.

Experiments on constancy of	Difference of percentages between + C and — C cases	
	within groups 2—3 "adaptable" phantoms	within groups 4—5 "rigid" phantoms
Brightness	15.5 ± 10 k = 1.6	11.5 ± 10.6 k = 1.1
Arm movement amplitude	18 ± 9.1 k = 2.0	21 ± 9.6 k = 2.2
Phantom weight .	25 ± 9.3 k = 2.7	38 ± 9.2 k = 4.1

Furthermore, a tentative hypothesis according to which the group of persons yielding low values of constancy have some common trait with those showing fixed phantoms and those yielding + C values with the adaptable phantom, may motivate the examination of perceptual data *within* groups of the — C or + C behaviour.

This is done in the following manner. The percentile distri-

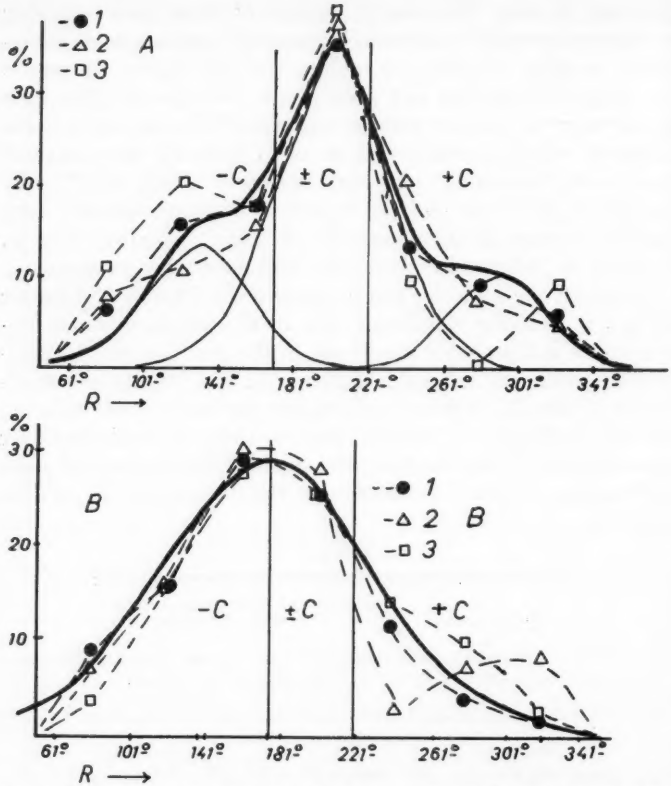

Fig. 5. Experiments on constancy of brightness. Percentile distribution of the R-values in different experimental groups A: 1 = "Adaptable" phantoms. 2. + C group of phantom weight. 3. Arm movement amplitude experiments with BR values > 0.65. Thin lines: Component binomial distribution curves fitted to the experimental data as to give when summated (heavy line) approximately the found distribution. B: 1. "Rigid" phantoms. 2. — C groups of phantom weight. 3. Arm movement amplitude experiments with BR values < 0.65. Heavy line: Binomial distribution curve approximating the experimental distribution.

bution of the values in the experiments on constancy of brightness is determined

a) in the group of cases with adaptable phantoms,

b) in the group of cases yielding relatively high values in the arm movement amplitude constancy (BR > 0.65),

c) in the cases with equality of subjective weight of the phantom and the intact arm (+ C group).

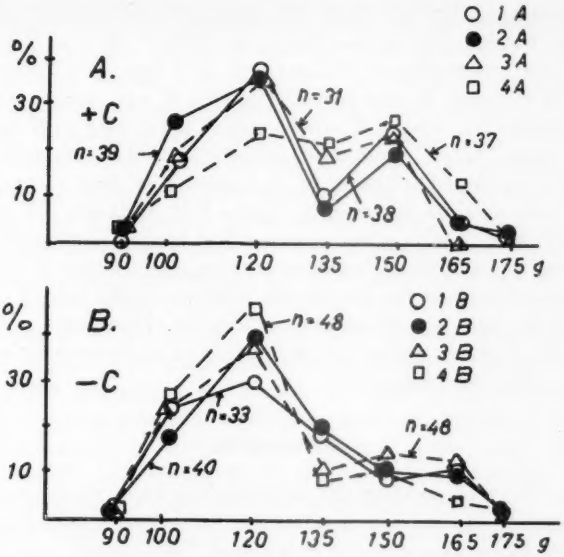

Fig. 6. Size-weight illusion in different experimental groups 1 A: Experiments on constancy of brightness. BR > 0.80. 2 A: "Adaptable" phantoms, groups 1—3. 3 A: + C group of phantom weight. 4 A: Arm movement amplitude constancy; BR values > 0.65. 1 B: Brightness experiments with BR values below 0.80. 2 B: "Rigid" phantoms, groups 4—5. 3 B: — C and ± C groups of phantom weight. 4 B: Arm movement amplitude experiments with BR values below 0.65.

The distribution curves are drawn in the same co-ordinate system (fig. 5 A) and below in figure 5 B are shown the corresponding distribution curves for "rigid"-phantom and — C groups. It is asked whether there is closer resemblance between the curves of figure 5 A or 5 B respectively than when curves of figure 5 A are compared with those of figure 5 B. This seems to be the case, and in a rather curious way. All curves of figure 5 B show up to fairly high constancy values (+ C range) a quite regular, normal distribution. In figure 5 A again, all curves are obviously abnormal in practically every region (except possibly the narrow range of intermediate values) and are suggestive of a composite structure of the series. This fact is illustrated by component probability ogives fitted as to give when summated, approximately the experimentally found distribution. In figure 5 B, as mentioned above, one binomial distribution curve covers nearly the whole range of values.

Encouraged by this result the percentile distribution of size-weight illusion values was studied as well. In spite of the small number of observations a similar trend, as in figure 5 is discernible. Figures 6 A and B give the distribution of equivalence weights in different + C and — C groups and in the groups with "adaptable" and "rigid" phantoms, respectively. As seen from the figure 6 A, the distribution in + C groups is clearly composite with two maxima whereas in figure 6 B although the curves are asymmetric with a tail at the end of greater illusion (heavier weights) the duplicity is not as striking as in figure 6 A. It is obvious that since the groups are small no single pair of curves differs enough for statistical significance. However, a similar trend in 4 curves makes the probability of occurrence by chance very much less likely.

Discussion.

The result of dividing the material into two groups according to whether the subject manifests a behaviour yielding ratings in the upper range of the postulated "constancy" scale or at the lower end of the same scale, can be summarized as follows. If the material is treated as one, the ratings show in a particular test a large, but fairly normal scatter around one maximal value. When, however, only those individuals are taken into account who gave high ratings in some of the constancy tests the distribution curves manifest abnormalities which can be resolved by supposing the existence of more than one (2 or 3) predilected values *i. e.* by dividing the distribution curve in 2 or 3 component probability ogives. In those subjects, on the other hand, who give in one test low "constancy" ratings the distribution of values corresponds more closely to the normal distribution curve. Thus, to put it short, the constancy experiments and the phenomenal characteristic of the phantom ("adaptable" or "rigid") are able to divide the subjects into two groups, one with a fairly uniform "intension pole" and a second with variable "intention poles".

The meaning of these "intention poles" may be discussed later in terms of the theory of perception of REENPÄÄ, which describes in exact terminology the texture of sensory experiments.

This interpretation might be too schematic and it is only tentative. As pointed out in the introduction, the conditions for

manifestation of constancy phenomena are the existence and notifying of cues which characterize the perceptual situation and secondly adaption of the percept to this situation. How the situation is conceived and consequently which adaptation of the percept is intended need not to be settled in this connection, but it may be pointed out that it may be a methodological question. Anyhow, when differences in the perceptual constancy appear they may depend either on the insufficiency of the cues for certain individuals or insufficient adaptability of the percept to the experimental situation. In those individuals who manifest perceptual constancy in some experiment an adaptation has obviously taken place and the recorded value is then indicative of the "ability" to adapt the percept to the "intended" thing. In another experiment the adaptability also exists but the "intention" may not be that corresponding to perceptual constancy. In those again, who yield in some experiments values of low "constancy" may be individuals who intend the stimulus value, but also those not "capable" to adapt the percept to the "objective" thing. The probability of occurrence of those capable of adaptation (but intending the "stimulus"-value) must therefore be less among those manifesting low constancy in some experiment.

On the other hand, if the phantom limb has adapted or adapts itself to a purely conceptual change in the perceptual situation as in the obstacle shunning experiment, the adaptability of the percept is evident. The group formed by these subjects is therefore comparable to those of the + C group, but it is not in any way determined which stimulus value is intended in any of the constancy experiments. The probability that these "adaptable" phantom carriers belong to the group that has yielded high constancy values is, however, greater than the probability that they belong in the group yielding low constancy values.

According to REENPÄÄ the stimulus-percept relationship is one of probability implication between abstraction classes formed on basis of equality; in symbols $E \underset{w}{\rightarrow} R$. To correspond the class (E) of equal perceptions an abstraction class (R) representing the stimulus is formed so as to show high probability (w) implication to the class E.

The elements of the stimulus class may represent whatever physical or chemical quantities, ratios, functions etc. the only determining condition being the correlation to the elements of the perception class. It is fairly obvious that success in finding

a stimulus is dependent upon the nature of the elements of the perception class. If the elements are simple one-dimensional sensations of intensity, time or place, the formation of an abstraction class of corresponding equal stimuli may be relatively easy. If, however, the elements of the perception class are highly integrated, "conceptualized", it may be almost impossible to find a class of equal quantities which would correspond to such percepts. In most constancy experiments including those of the present study, the percepts are highly conceptualized containing relations to intermodal past experiences etc. The elements of the perception class may therefore differ, in spite of equal instructions, in different individuals and a common class with high probability implication relation to these variable individual abstraction classes is not to be found. The broad-based probability ogive of figure 5 B may be regarded as one indication of this fact. On the other hand, the equality of percepts may even in complicated situations be based on selection of some relatively simple perceptual relation. Such selection of some distinct perceptual pattern as basis for equality, is what is meant with the expression "adaptation of the percept to an intention pole". It is to be expected that a distinct stimulus value (class) corresponding to this kind of perceptual abstraction class may be possible to find. The postulated narrow-based component ogives in figure 5 A might possibly illustrate this fact.

In these experiments the nature of the postulated "intension poles" is not evident. In other fields of perceptions the duality of stimuli corresponding to a uniform percept has been clearly shown. In discrimination of weights the duality can be resolved in skin pressure and muscle tension components (WANGEL et al. 1931, JALAVISTO 1935, JALAVISTO and BOMAN 1954). In estimating the push of a moving ball a shift from equality of force impulse, $m \cdot v$, to the equality of energy, $^1/_2 \cdot mv^2$, as basis of the perceptions, has been shown to occur without any phenomenal difference of the sensations (REENPÄÄ and BOMAN 1953).

It is not possible in this connection to discuss the various theories of perceptual constancy, of which the "probabilistic" (BRUNSWIK 1943) and the "transactional" (ITTELSON 1951, KILPATRICK 1952) theory have in the last years elicited most interest. (See also BLAKE and RAMSAY 1951, CANTRIL and coworkers 1949 and VERNON 1952, 1953.)

Summary.

1. Experiments on perceptual constancy in the fields of different sensory modalities were performed on arm amputees. (Constancy of brightness, constancy of arm movement amplitude, size-weight illusion, constancy of phantom arm weight.)

2. No intercorrelation was found between the ratings of the different tests.

3. The subjects were divided into two groups according to the behaviour of the phantom: those with "adaptable" and those with "rigid" phantoms.

4. It is shown that there are slightly more "rigid" phantoms in the groups of subjects with values corresponding to low constancy in the different tests (figure 4).

5. The distribution of the values indicating equality of brightness are examined in the groups with "adaptable" and "rigid" phantoms and a certain difference is noted. A tentative interpretation of the finding is given in terms of the perceptual theory of REENPÄÄ.

References.

BLAKE, R. R. and G. V. RAMSAY, (Eds) Perception: An Approach to Personality. The Ronald Press Co, New York, N. Y. 1951.

BOMAN, K. and E. JALAVISTO, Acta physiol. scand. 1954 (in the press).

BRUNSWIK, E., Wahrnehmung und Gegenstandswelt. Springer, Wien 1934.

— Psychol. Rev. 1943. *50*. 255.

CANTRIL, H., A. AMES, A. H. HARTORF, JR and W. H. ITTELSON, Science 1949. *110*. 461. 491. 512.

ITTELSON, W. H., Psychol. Rev. 1951. *58*. 285.

JALAVISTO, E., Acta Med. Fenn. "Duodecim" Ser. A. Tom. XVIII. 3. 1935.

— Duodecim 1942. *58*. 187. (Finnish.)

— Acta physiol. scand. 1946. *11*. 111.

— J. Gerontol. 1950. *5*. 339.

— K. LEPPÄNEN, M. SELVÄNNE, L. TAMMILEHTO and K. ÄIJÄLÄ, Scand. Arch. Physiol. 1937. *75*. 215.

— and P. SOURANDER, Ann. Acad. Sci. Fenn. 1948. A. V. 17.

KILPATRICK, F. P., Human Behaviour from the transactional point of view. Hanover N. H. Institute for Associated Research. 1952. Quoted by Psychol. Abstr. *27*. 1952.

RENQVIST-REENPÄÄ, Y., Allgemeine Sinnesphysiologie. Springer, Wien 1937.

Reenpää, Y., Über Wahrnehmen, Denken und Messendes Versuchen. Bibl. Biotheor. Leiden, Brill 1947.
— Sitzungsber. Heidelberger. Akad. Wiss. Math.-naturwiss. Kl. 1953.
— and K. Boman, Ann. Acad. Sci. Fenn. 1953. A. V. 39.
Thouless, R. H., In Essays in Psychology dedicated to David Katz. Uppsala 1951.
— Brit. J. Psychol. 1931. *22*. 1.
— Ibidem 1931. *22*. 216.
Vernon, M. D., A further study of visual perception. New York, Cambridge Univ. Press 1952.
— Ann. Rev. Psychol. 1953. *4*. 59.

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Observations Concerning the Absorption of Vitamin B_{12} by Cells of Escherichia Coli.

By

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The absorption of vitamin B_{12} by cells of *Escherichia coli* was demonstrated in 1951 by BURKHOLDER. HOFF-JÖRGENSEN, SKOUBY and GAD-ANDRESEN investigated in 1952 a total of 72 strains of *Escherichia coli* and they observed a considerable absorptive ability in the majority of investigated strains.

However, the mechanism of this phenomenon has as yet no explanation. The problems of this work have been:

1) Which part of the bacillary cell takes part in the absorption phenomenon?

2) Is the absorption only limited to metabolical functions of living cells?

The author isolated at first a strain of *Escherichia coli* from normal human feces. The strain was cultivated on stab agar cultures before use. It was then inoculated in a medium containing 10 g of vitamin-free casamino acids (Difco), 10 g of peptone (Difco), 0.5 g of sodium chloride, 10 g of ammonium sulphate and 10 g of lactose per litre of distilled water.

The cells were harvested after 48 hours' growth with a Sharpless supercentrifuge as aseptically as possible and suspended in a medium described by HOFF-JÖRGENSEN and coworkers.

This medium has the following composition: 0.5 g of sodium chloride, 10 g of dibasic potassium phosphate, 0.1 g of magnesium sulphate (7 H_2O), 1 g of ammonium sulphate, 10 mg of potassium cyanide, 0.5 g of sodium citrate (3 H_2O) and 4 g of asparagine per 800 ml of distilled water. The medium was sterilised in portions of 40 ml and before use 10 ml of 5 per cent solution of glucose was added aseptically to each portion.

The amount of bacteria in 1 ml of this suspension was counted microscopically using Buerker-Tuerk chambers. One ml of this suspension contained 3×10^9 cells per ml.

The site of absorption in cells of *Escherichia coli* was studied using sonic waves.

Ten ml of this suspension were treated for 10 minutes with Rayton 9 K C sonic oscillator, using an effect of 150 mV. About 90 per cent of the cells were destroyed, only 10 per cent surviving this procedure.

The cells were further centrifugated at 5,000 r. p. n. at freezing point and a cell-free supernatant was isolated from the cell bodies. In microscopical examination no living cells were found in this supernatant. The cell bodies were further suspended in the same amount of Hoff-Jörgensen medium in which they were before the sonic treatment.

In the following series of tests the author compared the ability of several bacterial preparations to absorb vitamin B_{12}. Into each of six sterilized centrifuge tubes 0.2 γ of vitamin B_{12} were added aseptically. One ml of the cell-free supernatant, obtained by the sonic treatment, was added into each of the first two tubes, to the third and fourth tubes was added 1 ml of the dead cell body suspension, and to the fifth and sixth tubes 1 ml of living cell suspension.

After 3 hours' incubation at 37° C the cells were further centrifugated. Then 0.5 ml of the supernatant was diluted to 50 ml with distilled water and the vitamin B_{12} content of this dilution was determined by the method described by BURKHOLDER, using *Escherichia coli* 113—3 as test organism.

The results of these tests were:

	Vitamin B_{12} in the tubes	Bacterial preparation used	Amount of vitamin B_{12} absorbed by the bacterial preparations
		Cell-free supernatant	
Tube No. 1	0.2 γ	1 ml	0.03 γ
Tube No. 2	0.2 γ	1 ml	0.02 γ
		Sonic treated cells. Strength of the suspension 3×10^9 cells per ml	
Tube No. 3	0.2 γ	1 ml	0.10 γ
Tube No. 4	0.2 γ	1 ml	0.11 γ
		Living cells. Strength of the suspension 3×10^9 cells per ml	
Tube No. 5	0.2 γ	1 ml	0.12 γ
Tube No. 6	0.2 γ	1 ml	0.12 γ

From the results of this experiment it was concluded that the cell cuticles (walls) of *Escherichia coli* were the absorbing component. The ability to absorb vitamin B_{12} was about the same in living cells and in cells destroyed by the sonic procedure. Active components taking part in this reaction were protected through this treatment.

Another experiment relating to this problem was carried out with acetone-killed cells of *Escherichia coli*.

The same strain of *Escherichia coli* was cultivated as described above and harvested with a Sharpless supercentrifuge. It was killed with acetone at $\pm 0°$ C. The acetone was filtered off and the bacteria were desiccated for 30 minutes at room temperature. The killed bacteria were then stored in a cold place. The experiments with acetone-killed cells were carried out with the technique described above. The amounts of acetone-killed cells added and the media used were as follows:

Medium	Dried bacteria added	Vitamin B_{12} added	Amount of Vitamin B_{12} absorbed by the cells
Tube No. 1 2 ml saline	10 mg	0.2 γ	0.02 γ
Tube No. 2 2 ml saline	20 mg	0.2 γ	0.04 γ
Tube No. 3 2 ml Hoff-Jörgensen medium	10 mg	0.2 γ	0.02 γ
Tube No. 4 2 ml Hoff-Jörgensen medium	20 mg	0.2 γ	0.05 γ

The incubation time in this experiment was 3 hours at 37° C. The amount of 10 mg of dried bacteria corresponded, when it was well shaken and all large conglomerants were dissolved to about 3.7×10^9 cells per ml diluted in 2 ml of saline solution.

In cup cultivation tests no growth of the bacillus was observed.

From the results in these experiments it seems obvious that the absorption of vitamin B_{12} by the *Escherichia coli* cells is also possible after the termination of cellular metabolism. HOFF-JÖRGENSEN, GAD ANDRESEN and SKOUBY maintain that the bacterial metabolism is a condition for this phenomenon. These tests, however, show that also dead cells have such an ability in this connection.

Summary.

In a series of tests the author has studied the ability of dead cells of *Escherichia coli* to absorb vitamin B_{12}. Contrary to earlier

observations, the author found that cells of *Escherichia coli* killed by sonic waves or acetone are capable for this absorption.

References.

BURKHOLDER, P., Science 1951. *114*. 478.
— Ibid. 1951. *114*. 459.
HOFF-JÖRGENSEN, E., A. P. SKOUBY, and J. GAD ANDRESEN, Nord. Med. 1952. *48*. 1754.

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Blood Eosinophil Responses to ACTH in Hyperemesis Gravidarum.

By

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There are several theories concerning the pathogenesis of hyperemesis gravidarum. From the end of the nineteenth century down to quite recent times the part played by psychogenic and neurogenic factors in the nausea and vomiting of early pregnancy has been repeatedly emphasized. When the significance of the adrenal cortex for the resistance of the body to diverse toxic agents and alarming stimuli of other kinds became known (BOINET 1896, DALE 1920, WYMAN 1928, SELYE 1936, and others) and it was found that the clinical symptoms and signs of hyperemesis of pregnancy resembled those of Addison's disease, the view gained ground that insufficiency of the adrenal cortex was responsible for the nausea and vomiting present in early pregnancy. Experiments with adrenal cortical preparations have given rather favourable results in hyperemesis (KEMP 1932, 1947, and many others), though less favourable results have been reported by BRANDSTRUP (1939) and LANGENDORFER (1950). ELERT (1940) called attention to the great differences noted in the metabolic changes of early pregnancy and those of late pregnancy, the changes of early pregnancy having a resemblance to those seen in relative adrenal insufficiency. BRANDSTRUP (1939), however, found little evidence of insufficiency of the adrenal cortex being responsible for hyperemesis gravidarum.

Several authorities have called attention to the fact that the chorionic gonadotropic hormone content of the blood and urine

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is increased in early pregnancy, particularly when hyperemesis is present (ANKER and LALAND 1934, BROWNE and VENNING 1936, EVANS and co-workers 1937, GENELL 1946, and others). YOUSSEF and BARSOUM (1953) emphasize the part played by an allergic factor in the pathogenesis of the vomiting of pregnancy.

HILLS and co-workers (1948) reported that the intramuscular injection of 25 mg of ACTH in patients with intact adrenals was followed by a rapid fall in the number of circulating eosinophils and lymphocytes. Prolonged administration of ACTH produces a sustained fall in the number of circulating eosinophils (PRUNTY 1950). Eosinopenia is a frequent finding in Cushing's syndrome (DE LA BALZE and co-workers 1946, and others). In patients with Addison's disease, on the other hand, ACTH produces no decrease in eosinophils, while cortisone does produce a decrease of this kind (THORN and co-workers 1947, DOUGHERTY and WHITE 1947, FORSHAM and co-workers 1948). Mild eosinophilia and relative lymphocytosis are often present in Addison's disease. The blood eosinophil count has therefore been taken as some sort of indicator of the endogenic pituitary-adrenal activity. THORN and co-workers (1948) proposed a test for adrenal cortical insufficiency, based on the response of the circulating eosinophils to ACTH.

The object of the investigation now reported was to study the responsiveness of the adrenal cortex to ACTH in hyperemesis gravidarum, with the eosinopenic response as a criterion.

The Present Investigation.

The series consisted of the cases treated in the Second Women's Clinic for hyperemesis during the first trimester of pregnancy. The control series consisted of normal pregnant women whose pregnancy had advanced equally far but who showed neither nausea nor vomiting. The normal subjects had been admitted for examination with a view to artificial termination of pregnancy.

The hyperemesis was of moderate severity. It subsided after the application of the combined therapy used in the clinic (rest, sedatives, infusions of glucose and saline, hormones). Thorn's test was carried out on the morning following the day of admission, before treatment was instituted.

This test was carried out as follows: On the morning, after 12 hours' fasting, 25 mg of ACTH (Cibachten, Ciba) was injected intramuscularly. The eosinophil count, using Randolph's (1944) stain, was done before the administration of ACTH and repeated 4 hours after it.

Results.

The results are shown in Table 1. In normal subjects the eosinophil count ranged between 40 and 500 per cu mm before the administration of ACTH, with an average of 216. By the second count, 4 hours after ACTH administration, the number of the eosinophils was 75.5 per cent smaller on an average, the decrease ranging between 50 and 93.7 per cent in the individual cases.

Table 1.

Changes in the Number of Circulating Eosinophils Following Administration of ACTH.

In Normal Pregnancy			In Hyperemesis Gravidarum		
Eosinophil Count Cells per cu mm		Percentile Change	Eosinophil Count Cells per cu mm		Percentile Change
Before ACTH	4 Hours after 25 mg of ACTH Intramuscularly		Before ACTH	4 Hours after 25 mg of ACTH Intramuscularly	
40	20	— 50.0	70	50	— 28.6
110	20	— 81.8	110	60	— 45.5
130	10	— 92.3	170	130	— 23.5
160	10	— 93.7	180	130	— 28.0
180	40	— 77.8	250	260	+ 4.0
190	60	— 68.4	330	140	— 57.6
200	40	— 80.0	340	230	— 32.7
310	70	— 77.4	710	250	— 64.8
340	70	— 79.4	840	510	— 39.3
500	190	— 62.0	900	350	— 61.1

In the cases of hyperemesis gravidarum the pre-ACTH number of eosinophils ranged from 70 to 900 per cu mm, with an average of 390. The post-ACTH development of the eosinophil count resulted in values which ranged from minus 64.8 per cent to plus 4 per cent, with an average of minus 37.7 per cent. In only three cases of this series did the fall in the eosinophil count exceed 50 per cent.

The results are illustrated by the graph in Fig. 1.

Discussion.

The initial eosinophil counts carried out on fasting patients before the administration of ACTH yielded somewhat higher

values in the hyperemesis series than in the control cases. The difference can hardly be accounted for by dehydration alone. It is worthy of note that a parallel difference in initial eosinophil counts between persons with Addison's disease and normal con-

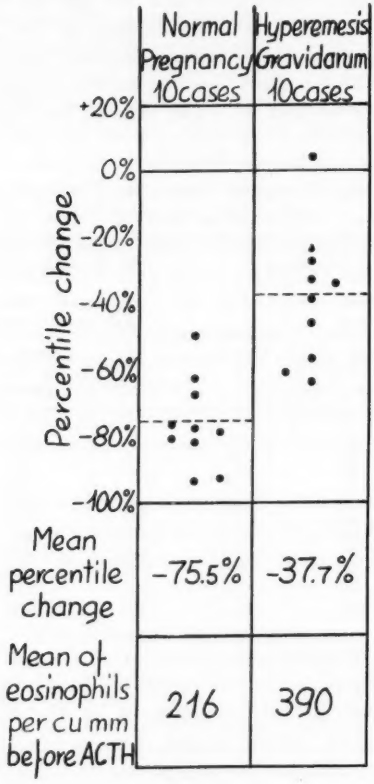

Fig. 1.

trols reported by FORSHAM and co-workers (1948) was not statistically significant either. It is obvious that factors other than adrenal cortical hormone output regulate the basal level of circulating eosinophils. Both in the hyperemesis series and control cases the basal eosinophil count fell only once below 100. The result shows that no noticeable eosinopenia is present in the

early phase of normal pregnancy. ANDREWS and BONSNES (1951), FERGUSON (1951), and KYANK and HASS (1953) found no abnormal variations in the basal eosinophil count during the whole period of pregnancy. BERNSTINE and ANDINO (1952) recorded no alterations in the number of circulating eosinophils even in toxemias of late pregnancy, while DAVIS and HULIT (1949) and DAWSON (1953) found a distinct fall in the blood eosinophil count in the course of normal pregnancy and especially during labour and eclampsia, with a return to normal after labour. DAVIS and HULIT assumed that the fall in the eosinophil count was due to increased secretion of 11-oxycorticosteroids and possibly also to the formation of steroids of the same type in the placenta. CATON and co-workers (1950) also reported a fall in the eosinophil count during pregnancy. KULLANDER (1952) found that the number of eosinophils decreased during the last week of normal pregnancy. SOIVA (1953) recorded no changes in the response of circulating eosinophils to ACTH in normal pregnancy nor in toxemias of late pregnancy, with the exception of the more severe forms of toxemia, in which the eosinophil response to ACTH tended to be stronger than normal.

A certain reservation is necessary in the evaluation of results obtained by Thorn's method. POSEY and co-workers (1950) believe that only a fall to less than 30 per cent is clearly pathological, while percentages ranging between 49 and 30 are inconclusive borderline values. When the classification proposed by POSEY and co-workers is applied to the present writers' results obtained with Thorn's method, the eosinophil response to ACTH was normal in all cases of normal pregnancy, while 4 cases of the hyperemesis series were pathological, 3 normal, and 3 uncertain borderline cases. The series examined was too small to allow any definite conclusions, but the result seems to suggest that subnormal eosinophil response to ACTH occurs more frequently in hyperemesis gravidarum than in those cases of pregnancy in which neither nausea nor vomiting is present. Considering the fact that 11-oxycorticosteroids have a marked eosinopenic action (PERERA and co-workers 1949, FOURMAN and co-workers 1949, RECANT and co-workers 1950, DWORETZKY and co-workers 1950, SOLOMON and SHOCK 1950, HIGGINS and co-workers 1951, QUITTNER and co-workers 1951), while desoxycorticosterone is ineffective in this respect (SPIES and STONE 1949, RECANT and co-workers 1950), it seems reasonable to assume that the condition

is due to a temporary interference in adaptation during which at any rate the absolute or relative output of 11-oxycorticosteroids is insufficient.

Summary.

Thorn's test was carried out, during the first trimester of pregnancy, in ten cases of hyperemesis gravidarum and in ten control cases of normal pregnancy.

In the cases of hyperemesis, the average initial eosinophil count carried out before the administration of ACTH was slightly higher than in the control series. After the injection of ACTH, 70 per cent of the cases of hyperemesis showed a fall of less than 50 per cent in the number of circulating eosinophils, while in all control cases it was more than 50 per cent.

The result seems to suggest the presence of an adaptation disturbance in hyperemesis gravidarum, with an insufficient absolute or relative output of 11-oxycorticosteroids.

Acknowledgement.

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References.

Andrews, W. C. and R. W. Bonsnes, Amer. J. Obstet. Gynec. 1951. *61.* 181.
Anker, H. and P. Laland, Acta Obstet. Gynec. Scand. 1934. *14.* 310.
Bernstine, J. B. and A. M. Andino, Amer. J. Obstet. Gynec. 1952. *63.* 181.
Boinet, E., C. r. Soc. Biol. 1896. *10.* 364.
Brandstrup, E., Acta Obstet. Gynec. Scand. 1939. *19.* 376.
Browne, J. S. L. and E. M. Venning, Amer. J. Physiol. 1936. *116.* 18.
Caton, W., D. E. Reid, C. E. Rath, C. A. Finch and L. Conroy, Surg. Gynec. Obstet. 1950. *90.* 320.
Dale, H. H., Brit. J. exp. Path. 1920. *1.* 103.
Davis, M. E. and B. E. Hulit, J. Clin. Endocrinol. 1949. *9.* 714.
Dawson, D. W., J. Obstet. Gynec. Brit. Emp. 1953. *60.* 727.
De la Balze, F. A., E. C. Reifenstein, Jr., and F. Albright, J. Clin. Endocrinol. 1946. *6.* 312.
Dougherty, T. F. and A. White, J. Lab. Clin. Med. 1947. *32.* 584.
Dworetzky, M., C. F. Code and G. M. Higgins, Proc. Soc. Exp. Biol. Med. 1950. *75.* 201.

ELERT, R., Klin. Wschr. 1940. *19*. 49.
EVANS, H. M., C. L. KOHLS and D. H. WONDER, J. A. M. A. 1937. *108*. 287.
FERGUSON, J. H., Amer. J. Obstet. Gynec. 1951. *61*. 603.
FORSHAM, P. H., G. W. THORN, F. T. G. PRUNTY and A. G. HILLS, J. Clin. Endocrinol 1948. *8*. 15.
FOURMAN, P., F. C. BARTTER, F. ALBRIGHT, E. DEMPSEY, E. CARROL and J. ALEXANDER, J. Clin. Invest. 1950. *29*. 1462.
GENELL, S., Acta Obstet. Gynec. Scand. 1946. *26*. 555.
HIGGINS, G. M., K. A. WOODS and E. C. KENDALL, Endocrinology 1951. *48*. 175.
HILLS, A. G., P. H. FORSHAM and C. A. FINCH, Blood 1948. *3*. 755.
KEMP, W. N., Endocrinology 1932. *16*. 434.
— Canad. Med. Ass. J. 1947. *56*. 409.
KULLANDER, S., Acta Endocr. Copenhagen 1952. *10*. 135.
KYANK, H., and H.-D. HASS, Arch. Gynäk. 1953. *182*. 728.
LANGENDORFER, G., Ärztl. Wschr. 1950. *5*. 287.
PERERA, G. A., K. L. PINES, B. HAMILTON and K. VISLOCKY, J. Clin. Invest. 1949. *28*. 803.
PERERA, G. A., K. L. PINES, B. HAMILTON and K. VISLOCKY, Amer. J. Med. 1949. *7*. 56.
POSEY, E. L., D. R. MATHIESSON, H. L. MASON and J. A. BARGEN, Amer. J. Med. Sci. 1950. *219*. 651.
PRUNTY, F. T. G., J. Clin. Path. 1950. *3*. 87.
QUITTNER, H., N. WALD, L. N. SUSSMAN and W. ANTOPOL, Blood 1951. *6*. 513.
RANDOLPH, T. G., J. Allergy 1944. *15*. 89.
RECANT, L., D. M. HUME, P. H. FORSHAM and G. W. THORN, J. Clin. Endocrinol. 1950. *10*. 187.
SELYE, H., Amer. J. Physiol. 1936. *116*. 141.
SOIVA, K., Acta Endocr. Copenhagen 1953. *12*. 90.
SOLOMON, D. H. and N. W. SHOCK, J. Gerontol. 1950. *5*. 302.
SPIES, T. D. and R. E. STONE, Lancet 1949. *2*. 890.
THORN, G. W., P. H. FORSHAM, F. T. G. PRUNTY, G. E. BERNER and A. G. HILLS, Ann. N. Y. Acad. Sci. 1949. *50*. 646.
THORN, G. W., P. H. FORSHAM, F. T. G. PRUNTY and A. G. HILLS, J. A. M. A. 1948. *137*. 1005.
THORN, G. W., F. T. G. PRUNTY and P. H. FORSHAM, Trans. Ass. Amer. Physcns. 1947. *60*. 143.
WYMAN, L. C., Amer. J. Physiol. 1928. *87*. 29.
YOUSSEF, A. F. and G. S. BARSOUM, J. Obstet. Gynec. Brit. Emp. 1953. *60*. 388.

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Permeability of Placenta of the Guinea Pig to Glucose and Fructose.

By

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Fructose, in addition to glucose, is present in the foetal blood of ungulates at a considerable concentration, whereas the maternal blood of these animals contains no fructose (COLE and HITCHCOCK 1946, BACON and BELL 1948, PARTRIDGE 1948, HITCHCOCK 1949). On the other hand, no — or practically no — fructose has so far been found in the foetal blood of other mammals examined (KARVONEN 1949a, AINSWORTH et al. 1951, GOODWIN 1952, HAGERMANN and VILLEE 1952).

Fructose is produced in the placenta, as has been shown on sheep (HUGGETT et al. 1949, 1951, KARVONEN 1949b, ALEXANDER et al. 1951, 1952). Fructose remains in the foetal circulation owing to a relative impermeability of placenta to fructose in comparison to glucose (KARVONEN 1949b, HUGGETT et al. 1951). The different behaviour of the two sugars necessitates the assumption that at least one of them, evidently glucose, is transferred through the placenta by an "active" mechanism, the kinetics of which have been discussed by WIDDAS (1952). This refutes an earlier theory (*e. g.* ANSELMINO 1929, BRANDSTRUP 1929, SCHLOSSMAN 1932), according to which the permeability of placenta is entirely referable to the physical laws of diffusion and osmosis.

The permeability of glucose and fructose through the placenta has not previously been compared, so far as the writers are aware, in any species in which the foetal blood contains no fructose. To do this in a rodent, the guinea pig, was the purpose of the present study. The results showed that the different permeability

of glucose and fructose through placenta is not limited to ungulates only, but appears to be a characteristic more or less common also to other mammals.

Material and Methods.

Guinea pigs were used as experimental animals. They were anaesthetized with "Nembutal" intraperitoneally. The foetuses were delivered through caesarean section into a 0.9 % NaCl bath kept at + 38° C, where they remained connected with a functioning placenta (HUGGETT 1927). The foetuses were weighed after the experiment; their exact age was not known.

Two kinds of experiments were performed. In one type 5 or 10 % (weight/volume) glucose or fructose solution was infused in the maternal animal with the aid of a constant speed infusion pump, at a speed of 0.417 ml (= 21 or 42 mg) per min. In the other type, 0.5 or 1.0 ml of the corresponding solutions was injected — not infused — into the foetal circulation. In both kinds of experiments, successive blood samples were taken as well from the mother as from the foetuses.

For infusions into the maternal animal, the jugular vein or the carotid artery was cannulated with polythene tubing. Blood samples were taken through another polythene cannula from the carotid artery of the opposite side, keeping it open meanwhile with the aid of heparine.

The injections into the foetal circulation were made, slowly, into the umbilical vein, and foetal blood samples were also taken from the same, puncturing it each time.

One ml blood was taken at a time from the mother and 0.5 ml from each foetus. The blood was deproteinized with the cadmium precipitation (FUJITA and IWATAKE 1931). The total reducing substance was determined according to SOMOGYI (1945) and NELSON (1944), taking the readings with a Coleman electrical spectrophotometer, at 5,200 Å. The fructose concentration was estimated with COLE's modification (BACON and BELL 1946, KARVONEN and SOMERSALO 1949) of ROE's (1934) colorimetric method. The readings were taken with a Coleman spectrophotometer, at 4,700 Å. The analyses were made in duplicate. A series of glucose and fructose standards representing the expected range of blood sugar concentration was included in each batch.

The duration of the experiments was limited by the amount of blood available in the foetuses. The substantial loss of blood due to sampling may have brought the foetuses into an unphysiological state, but as all the foetuses were subjected to the same produce, this will not essentially affect the comparative results.

Results.

Transfer of glucose from mother to foetus. Three experiments were performed. The foetal blood sugar, which before the infusion had been close to the maternal value, rose during the infusion

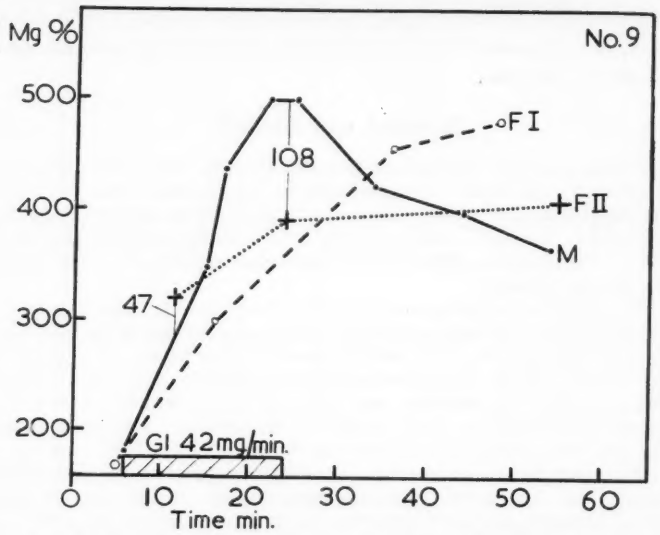

Fig. 1. Infusion of glucose into the mother (M). The glucose level in the foetal blood (F I and F II) rises closely parallel with the maternal rise. The thin vertical lines indicate the M-F gradients used for the calculation of the "diffusion constant".

closely parallel with the rise of the maternal glucose. An example of the type of results obtained is given in Fig. 1. It was obvious that the placenta was freely permeable to glucose as coming from the maternal side.

In four cases two successive samples were obtained from a foetus during the infusion — or the second sample immediately after the end of the infusion — (Table: A). In order to express the rate of the rise of the foetal glucose level the difference between the two successive foetal glucose values was divided by the interval of sampling in minutes. For relating the rate to the maternal glucose level, it was divided by the mean mother-foetus (M-F) glucose gradient (in mg-%) during the period of observation. This again was obtained as follows: for each foetal glucose value, the simultaneously prevailing maternal blood glucose level was obtained with the aid of interpolation from the two nearest determinations; each foetal glucose value was subtracted from the corresponding maternal (interpolated) value, and the mean of these two gradients was taken as the mean gradient prevailing during the interval of the two successive foetal samples.

Table.

The placental transfer of glucose and fructose.

No of experiment and foetus	Foetal weight gm	Interval of samples min	Change of foetal sugar mg%	Rate of change mg% per min	Mean M-F gradient mg%	*Change* time × gradient
A. Glucose: mother → foetus:						
4 F II	95	17	238 → 426 = 242	14.2	63.5	0.272
6 F I	81	21	294 → 351 = 57	2.7	23	0.118
9 F I	61	11	169 → 298 = 129	11.7	50	0.234
F II	75	13	321 → 390 = 69	5.3	30.5	0.174
					Mean ± SD:	0.200 ± 0.068
B. Glucose: foetus → mother:						
7 F II	46	15	659 → 378 = 281	18.8	300.5	0.062
11 F III	65	8	319 → 149 = 170	21.2	91	0.234
12 F III	49	11	352 → 282 = 70	5.6	83.5	0.075
F III		14	282 → 258 = 24	1.7	33	0.052
4 F I	67.5	19	382 → 315 = 67	3.5	47	0.075
					Mean ± SD:	0.100 ± 0.076
C. Fructose: mother → foetus:						
5 F II	?	35	12 → 43 = 31	0.9	124	0.0071
F III	?	19	17 → 42 = 25	1.3	127	0.0104
10 F I	57	11	2 → 13 = 11	1.0	76	0.0060
F II	69	13	11 → 24 = 13	1.0	86	0.0063
F III	64	15	17 → 32 = 15	1.0	124	0.0037
					Mean±SD:	0.0067 ± 0.0025
D. Fructose: foetus → mother:						
7 F I	46	17	346 → 290 = 56	3.3	318	0.0104
11 F I	81.5	13	182 → 136 = 46	3.5	159	0.0222
12 F II	44	22	214 → 198 = 16	1.4	203.5	0.0036
					Mean±SD:	0.0121 ± 0.0094

The obtained expression: $\frac{\text{Change in foetal blood sugar level,}}{\text{Time (min)} \times \text{M-F gradient}}$ is analogous to the constant describing physical diffusion and may be used as such, if the change in the foetal blood sugar level is considered as representing the amount of sugar in the foetal space.

The constant as calculated for the above experiments is shown in the Table. The range was from 0.118 to 0.272 and the mean 0.200 ± 0.068 (SD) (mg-% per minute per mg-% M-F gradient).

Transfer of glucose from foetus to mother. Glucose was injected into the umbilical vein of three foetuses. The level of glucose fell fairly rapidly to that of the mother. An example is shown in Fig. 2, which represents an experiment where glucose was injected into one of the foetuses and fructose into another. The "diffusion

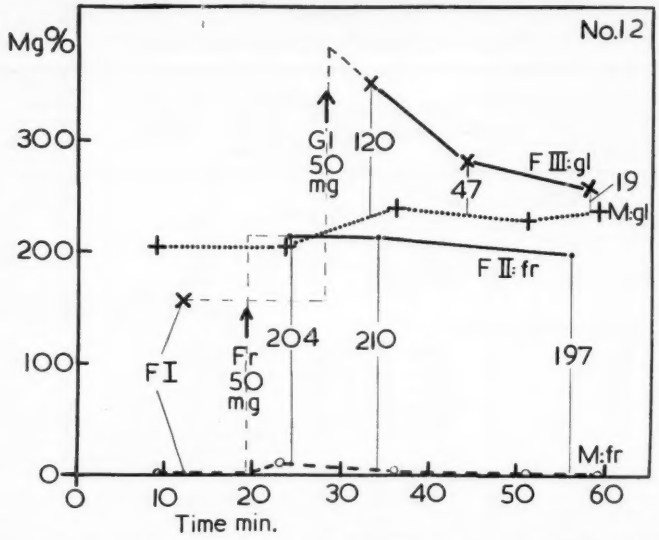

Fig. 2. Injection of 50 mg fructose into foetus II (F II) and 50 mg glucose into foetus III (F III). The fructose level in F II remains very stable, whereas the glucose in F III rapidly falls towards maternal level (M:gl). After the fructose injection, small amounts of fructose appear in the maternal circulation (M:fr). Thin lines as in Fig. 1. Glucose level of F II is left out for clarity.

constant" varied in these experiments from 0.052 to 0.234 with the mean at 0.100 ± 0.076 (SD) (Table: B).

After an infusion of glucose into the mother had stopped, the foetal blood glucose tended to be higher than the maternal value at the same time. From a total of ten foetal samples collected after stopping glucose infusion, seven were distinctly above the maternal level (by 97, 67, 53, 41, 39, 27, and 14 mg-%).

In one experiment (No. 4, FI) two successive samples were taken from the same foetus, while the maternal and foetal blood glucose levels were falling after a glucose infusion, the foetal glucose level being the higher one at that stage. The "diffusion constant" from the foetus to the mother was in this case only 0.075. In one case (No. 9, FI: Fig. 1), a rise of the foetal glucose level was observed after the end of the infusion, while the maternal glucose was at a lower level. However, because of the delicate nature of the mother-foetus preparation, too much weight must not be laid on a single observation.

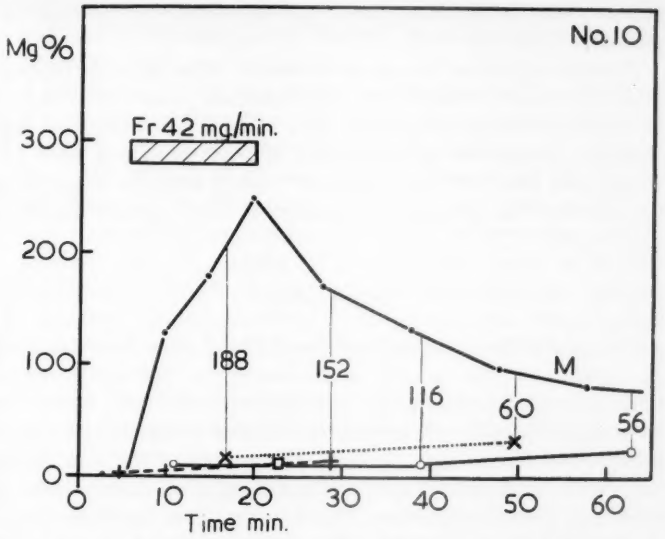

Fig. 3. Infusion of fructose into the mother (M). There is only a minimal rise of the foetal fructose. Samples were obtained from four foetuses, each marked in a different way. Thin lines as in Fig. 1. The glucose levels are left out for clarity.

With one exception, the diffusion constant was lower for the transfer from foetus to mother (F → M) than in the opposite direction (M → F). However, owing to the small number of experiments and to the relatively great scatter, the difference between the mean values of the constant — 0.200 for M → F and 0.100 for F → M — is not statistically significant ($t = 2.05$; $0.1 > p > 0.05$).

Transfer of fructose from mother to foetus. In four experiments, fructose was infused into the maternal circulation. Whereas in the glucose experiments the foetal glucose level closely followed the maternal one, the placenta proved very much less permeable to fructose. Fructose appeared only at relatively low concentrations in the foetal circulation. A typical example is shown in Fig. 3.

In five cases, two successive samples were obtained from the same foetus, while fructose was present in the maternal circulation. The rate of the rise of the foetal glucose level and the "diffusion constant" were calculated as above (Table: C). The "diffusion constant" varied from 0.0037 to 0.0104, with the mean at 0.0067 ±

0.0025 (SD), being thus 1/30 of the corresponding constant for glucose.

Transfer of fructose from foetus to mother. After injecting fructose into the foetal circulation, its concentration remained at a high level for a relatively long time. Fig. 2 shows an example of this, with the lowest rate of elimination of fructose among these experiments. The "diffusion constant" varied over the same range as that for the transfer of fructose in the opposite direction (Table: D).

Discussion.

Changes in the maternal and foetal blood sugar levels depend, in addition to the amount administered, on placental transfer and on loss or gain through extraplacental channels. If the loss of a sugar through extraplacental channels is relatively rapid in comparison to the gain through placenta from the side of administration, the resulting rise of blood sugar will be considerably less than the transfer alone would cause. This obviously is the case with fructose. If, on the other hand, the transfer is relatively rapid in comparison to the utilization, as with glucose, the resulting changes in blood sugar level are a more true indicator of the rate of transfer.

It is possible that minor differences exist in the rate of utilization of each of these two sugars in the mother and foetus, respectively (cf. KARVONEN 1949b), but under the conditions of the present experiments, when relatively large amounts of sugar are administered within a short time, the differences in the rate of utilization are not likely seriously to affect the type of results. Therefore, it may be concluded that the blood sugar levels obtained in the present study are reasonably reliable indicators of placental transfer.

Accordingly, the results demonstrate conclusively that the transfer of fructose through the placenta is considerably slower than that of glucose, independent of the direction of transfer. The mean constant for all the glucose experiments was 0.144 ± 0.086 (SD) and for all the fructose experiments 0.0087 ± 0.0060 (SD); the difference of these two average values is statistically significant ($t = 4.44$, $p < 0.001$). Thus, the permeability of the placenta of the guinea pig to these two sugars is comparable to that of sheep placenta. This again indicates that the permeability

of at least one of these sugars, evidently that of glucose, is determined also in this species by "active" processes and not merely by the laws of physical diffusion.

A comparison of the rate of the transfer from mother to foetus and vice versa suffers from the small number of experiments However, it appears that there is no marked difference for fructose whereas the transfer of glucose from the foetus may possibly be slower than in the "physiological" direction, from mother to foetus. Further data are needed of settling this problem.

In any case, the transfer of glucose from foetus to mother appears also to be an "active" process. The difference between the constant for this transfer, 0.100 ± 0.076, and the constant for fructose, 0.0087 ± 0.0060, is statistically significant ($t = 3,46$; $0.01 > p > 0.001$).

In sheep, the kinetics of the transfer of glucose from mother to foetus are compatible with the operation of a carrier system (WIDDAS). WIDDAS has pointed out that at high blood glucose levels the rate of transfer is relatively slow in comparison to lower ones, which is in accordance with the theoretically expected behaviour of a carrier system. However, the present series fails to show a comparable relationship; the "diffusion constant" seems to vary independently of the glucose level within the range of the observations. All glucose values, on the other hand, were relatively high. For a carrier system, a "diffusion constant" as calculated above has, of course, no real meaning, but it remains for further work to establish the characteristics of the carrier system operating in this transfer. If the rate of the transfer of glucose, moreover, proves to be different in the two directions, the carrier system will have to work in a "directional" way.

In the present results, there is nothing which would suggest the presence of a carrier system for fructose. However, further work may still prove this tentative conclusion to be wrong.

Summary.

The permeability of the placenta of guinea pig to fructose and glucose was studied *in situ*, by infusing the sugar into the maternal circulation or by injecting it into the foetal one.

The transfer of fructose in either direction was very much slower than that of glucose. For glucose, there was some suggestion

of a more efficient transfer from mother to foetus than vice versa, but this point needs further study.

It was concluded that the transfer of glucose through placenta — in both directions — is an "active" process in the guinea pig, similarly as it is known to be in the sheep, in spite of marked differences in carbohydrate metabolism between these two species.

References.

AINSWORTH, I. B., C. W. PARR and F. L. WARREN, J. Endocrin. 1951. *7*. LXIII.

ALEXANDER, D. P., R. D. ANDREWS, A. ST. G. HUGGETT, D. A. NIXON and W. F. WIDDAS, J. Physiol. 1952. *118*. 58 P.

— A. ST. G. HUGGETT and W. F. WIDDAS, Ibidem 1951. *115*. 36 P.

ANSELMINO, K. J., Arch. Gynäk. 1929. *138*. 710.

BACON, J. S. D. and D. J. BELL, Biochem. J. 1946. *40*. XLII.

— — Ibidem 1948. *42*. 397.

BRANDSTRUP, E., Acta Obst. Gynec. scand. 1929. *8*. 490.

COLE, S. W. and M. W. S. HITCHCOCK, Biochem. J. 1946. *40*. 51.

FUJITA, A. and D. IWATAKE, Biochem. Z. 1931. *242*. 43.

GOODWIN, R. F. W., Nature 1952. *170*. 750.

HAGERMANN, D. D. and C. A. VILLEE, J. clin. Invest. 1952. *31*. 911.

HITCHCOCK, M. W. S., J. Physiol. 1949. *108*. 117.

HUGGETT, A. ST. G., Ibidem 1927. *62*. 373.

— F. L. WARREN and V. N. WINTERTON, Nature 1949. *164*. 271.

— — and V. N. WARREN, J. Physiol. 1951. *113*. 258.

KARVONEN, M. J., Acta paediatr. 1949a. *37*. 68.

— Ann. Med. exp. Biol. fenn. 1949b. *27*. 197.

— and O. SOMERSALO, Ibidem 1949. *27*. 30.

NELSON, N., J. biol. Chem. 1944. *153*. 375.

PARTRIDGE, S. M., Biochem. J. 1948. *42*. 238.

ROE, J. H., J. biol. Chem. 1945. *160*. 161.

SCHLOSSMAN, H., Erg. Physiol. 1932. *34*. 744.

SOMOGYI, M., J. biol. Chem. 1945. *160*. 61.

WIDDAS, W. F., J. Physiol. 1952. *118*. 23.

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The Effect of Certain Choline Esters on the Chemoceptor Activity in the Carotid Body.

By

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Recent work has called attention to the possible rôle of acetylcholine or some closely related substance for the initiation of impulses in the chemoceptors of the carotid body (LILJESTRAND 1951, LANDGREN, LILJESTRAND and ZOTTERMAN 1952, 1954). In continuation of our earlier studies on this subject we have compared the effects of close arterial injection of choline esters on the action potentials in the sinus nerve deriving from the chemoceptors of the carotid body. In certain experiments the injections of the choline esters were preceded by the administration of some strong inhibitor of the cholinesterases.

Methods.

In all 19 cats were used. The animals were anesthetized with chloralose (0.07 g per kg) and urethane (0.25 g per kg) and artificially ventilated with pure oxygen. The general technique for recording action potentials from the sinus nerve has been described in our earlier papers. The substances, dissolved in Ringer's fluid, were injected through a special T-shaped cannula in the common carotid artery, close to the carotid sinus. This cannula also permitted recording of the blood pressure inside the carotid artery. Thus rapid injections were signalled by a brief rise in carotid pressure. 1 ml or sometimes less was injected in about $^1/_3$ of a second.

The following choline esters were tested:
Acetylcholine chloride (Roche),
Acetylthiocholine iodide (Roche),

Propionylcholine iodide,
Butyrylcholine iodide,
Butyrylthiocholine iodide,
Benzoylcholine chloride (Roche),
Acetyl-β-methylcholine iodide,
Succinylcholine iodide.

The substances with an inhibitory effect on cholinesterases employed were:

Diisopropoxyphosphoryl-fluoride (DFP), a stock solution in propylene glycol being diluted with Ringer,
Diethyl-p-nitrophenyl phosphate (Mintacol), the watery solution being similarly diluted,
Diiodomethylate of bis-(2-diethylaminoethyl)para-phthalate (302 I.S.),
Diiodomethylate of bis-(2-diethylaminoethyl)ortho-phthalate (306 I.S.).[1]

Since we wished to inactivate the cholinesterases of the carotid body without influencing those of the circulating blood it was necessary to bring the inhibitory substance in close contact with the chemoceptive organ for a short while without allowing it unnecessarily to enter the general circulation. A second cannula was therefore introduced into the lingual artery, the carotid was temporarily clamped above and below the carotid sinus and the solution of the substance injected into the carotid sinus and allowed to escape through the lingual cannula. The system was then closed for some minute and afterwards washed with Ringer's solution before the circulation was re-established. This procedure had also the advantage that general effects on the circulation could be avoided almost completely, whereas an intravenous injection would have led to a potentiation of the cholinergic effect on blood pressure and also to an awakening of the animal.

Results.

The immediate result of an injection through the carotid cannula is a local rise in the pressure within the carotid artery, and this is accompanied by an outburst of baroceptive impulses (Cp. Fig. 4 C). When the injection is ended the pressure returns to the original value in one or two heart beats. If Ringer solution alone is injected, a small transient decrease of the chemoceptive impulses is usually observed in this second phase. A stimulating effect of the chemoceptors from choline esters given in the Ringer solution may easily be masked by the baroceptor discharge during this initial phase of increased pressure, but if it persists after the

[1] We are indebted to HOFFMANN-LA ROCHE for supplies of benzoylcholine and acetylthiocholine, to Professor D. BOVET and Doctor F. BOVET-NITTI for the substances 302 I.S. and 306 I.S. and to the Research Institute of National Defence, Stockholm, for butyrylcholine, butyrylthiocholine, propionylcholine, acetyl-β-methylcholine as well as DFP and mintacol.

injection it can be observed in the following period. The response varies considerably with the dose and the substance injected. It may be rather insignificant and last only for some tenths of a second, in other cases it can still be traced after several seconds, and it sometimes happens that it starts some time after the end of the injection and gains its full strength only after a few seconds. In spite of the small doses used, a fall of blood pressure often occurred a short while after the injection when the substance entered the general circulation and caused vasodilation. This was especially the case after choline esters known to have a pronounced muscarine-like effect (*e. g.* acetyl-, propionyl-, butyryl-choline) or that had to be given in comparatively larger doses (benzoyl- and acetyl-β-methyl-choline) but only very little after the small amounts necessary of the thiocholines which have mainly a nicotine-like effect. The fall in blood pressure sometimes obscured the results, since the discharge of small diameter pressure afferents may become reduced; on the other hand the impairment of the blood flow through the carotid body may cause some local oxygen want and lead to a corresponding increase in the activity of the chemoceptors. In order to be able to draw conclusions regarding the chemical action potentials it is therefore desirable to compare sections of the recordings before and after the injection when the blood pressure was about the same. Sometimes controls had to be made by raising the blood pressure with adrenaline or dextrane.

The Direct Effect of Different Choline Esters.

The intracarotid injection of 0.5—1 μg of acetylcholine usually gave no stimulation of the chemoceptors or in some instances only a threshold response. When the dose was raised to 2—5 μg, a moderate effect was regularly observed lasting about 1—3 seconds. 5—10 μg caused a rather strong stimulation but it was still maintained only for the same short time (Fig. 1 A and B, compared to C). These results are in good agreement with those obtained by v. Euler, Liljestrand and Zotterman (1941).

The picture after propionylcholine or butyrylcholine was very similar to that following acetylcholine (Fig. 1 D and E), though in most cases the effect was somewhat more pronounced with acetylcholine. There were individual variations with regard to the relative activity of these two esters. Usually, but not always,

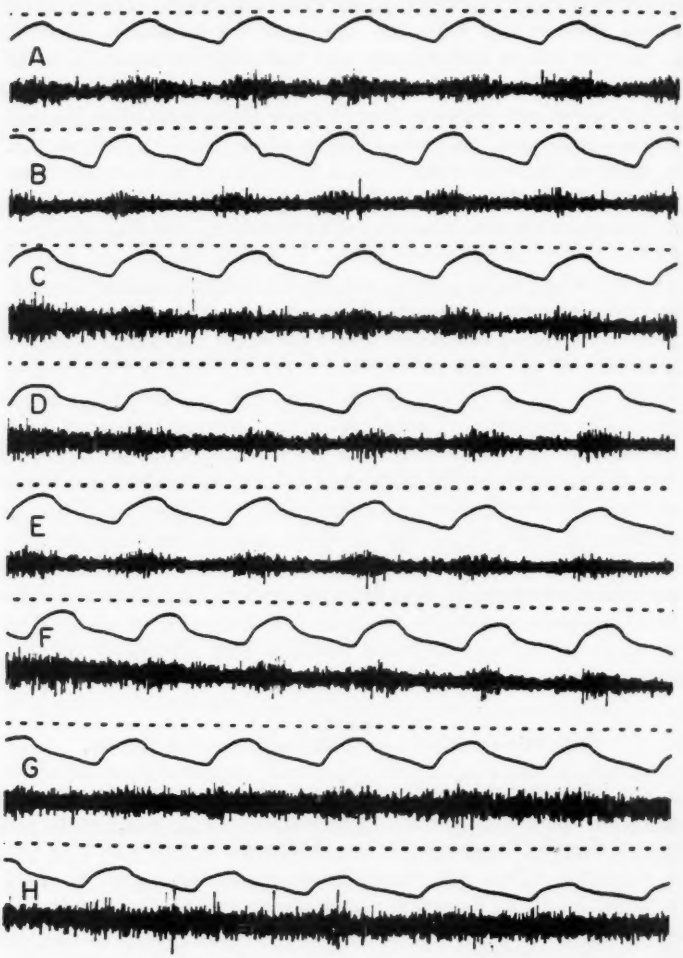

Fig. 1. All figures show the impulse activity from the carotid sinus nerve of the cat. Blood pressure is recorded in the external carotid. Time markings 0.04 seconds.

Artificial respiration with oxygen. MBP 160—180 mm Hg. A control. B —H 0.8 seconds after the injection of B 1 μg acetylcholine, C 10 μg acetylcholine, D 10 μg propionylcholine, E 10 μg butyrylcholine, F 100 μg benzoylcholine, G 10 μg acetylthiocholine and H 10 μg butyrylthiocholine.

propionylcholine acted a little more powerfully than butyrylcholine. With benzoylcholine the dose had to be 5—10 times greater than of acetylcholine in order to give the same stimulation (Fig. 1 F). For acetyl-β-metylcholine and succinylcholine still larger doses were necessary.

Acetylthiocholine was sometimes even more potent than acetylcholine, but in other experiments it displayed the same or even a somewhat smaller effect. It was characteristic, however, that the effect lasted much longer, usually 6—8 seconds after 5—10 μg (Fig. 1 G). Often the discharge started 0.5—1 second after the end of the injection and reached its full strength a second or so later — in contrast to acetylcholine where the greatest response was usually immediately after the injection and followed by a rapid decay.

Butyrylthiocholine was decidedly more active than butyrylcholine though probably not quite as active as the corresponding acetylcholine derivative, but both thioderivatives showed a similar striking prolongation of the discharge (Fig. 1 H).

The Effects of Pretreatment with Anticholinesterases.

1. Mintacol.

A solution containing 100 μg of mintacol per ml was brought into contact with the carotid body as described above for about 1 minute. As a result of this treatment 1 μg of acetylcholine was now as active (Fig. 2 A) as 10 μg before the application of mintacol and, in addition, the effect was prolonged to over 4 seconds. 10 μg of propionyl- or butyrylcholine which before had a very slight or even, in the case of butyrylcholine, a doubtful effect, now elicited very strong responses (Fig. 2 B and C). A corresponding increase was observed with 100 μg benzoylcholine (Fig. 2 D).

On the other hand acetylthiocholine and butyrylthiocholine both of which in a dose of 10 μg exercised a fairly strong action before mintacol was given, now produced considerably weaker effects (Fig. 2 E and F).

A second similar treatment of the carotid body with mintacol further reduced the response to 10 μg acetylthiocholine, whereas 1 μg acetylcholine had the same strong effect as after the first application of mintacol.

We also tried whether ventilation with air or with 10 per cent oxygen instead of pure oxygen would lead to a stronger stimulation

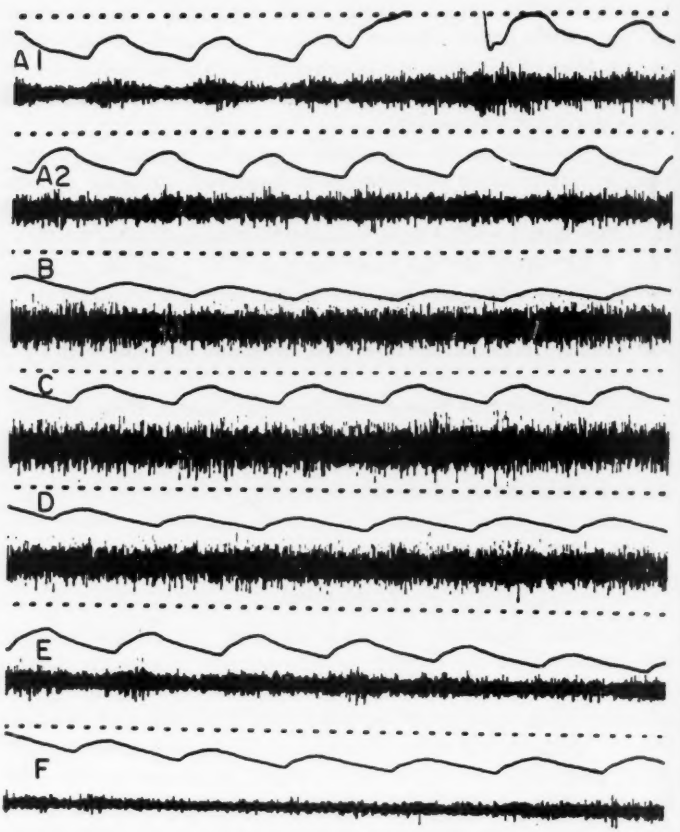

Fig. 2. Same preparation as in Fig. 1, but after local application of 100 μg per ml mintacol. MBP 160—170 mm Hg. A 1 injection of 1 μg acetylcholine, A 2 direct continuation of A 1, B—F 0.8 seconds after the injection of B 10 μg propionylcholine, C 10 μg butyrylcholine, D 100 μg benzoylcholine, E 10 μg acetylthiocholine and F 10 μg butyrylthiocholine.

than before, but practically no difference was found between the two series of observations.

Quite similar results with the choline esters were obtained in another animal when a stronger solution was used, containing 500 μg mintacol per ml. In this case, however, oxygen lack seemed to induce a somewhat increased response by comparison with

Fig. 3. Artificial ventilation with oxygen. A control, MBP 120 mm Hg, B after local application of mintacol (see text), MBP 80 mm Hg, C after mintacol and intravenous injection of 5 μg adrenaline, MBP 190 mm Hg.

that obtained before the drug had been administered. This stronger mintacol solution was for a second time applied, and now an enormous increase of the electrical activity appeared even during ventilation with pure oxygen (Fig. 3 A and B). Since the blood pressure had dropped to 80 mm Hg it might be argued that the effect was due to impairment of the local blood flow. The blood pressure was therefore raised to 190 mm Hg by intravenous injection of 5 μg of adrenaline. As was to be expected some reduction of the impulse traffic took place, but there was still a very large increase above the level before mintacol (Fig. 3 C) — in fact it was now about as large during oxygen ventilation as it was during ventilation with 10—15 per cent oxygen before.

It was also interesting to observe that the well-known stimulating effect of lobeline on the discharge from the carotid body was unaffected by mintacol.

2. DFP.

After local application of a roughly 3 to 6 mg per ml solution of DFP a considerable sensitization of the chemoceptors resulted. Before this treatment 5 μg of acetylcholine evoked the usual fairly strong response (Fig. 4 A). After DFP 1 μg led to a much greater increase which lasted for more than 6 seconds (Fig. 4 B). In another animal the effect was potentiated about 10 times. For butyrylcholine 5 μg gave only a very slight stimulation before

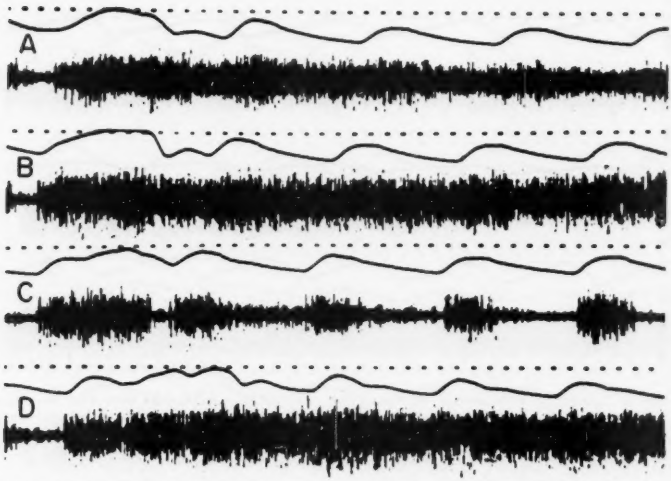

Fig. 4. Artificial ventilation with oxygen. MBP about 140 mm Hg. A injection of 5 μg acetylcholine (before DFP), B injection of 1 μg acetylcholine (after local application of DFP — 3 mg per ml), C injection of 5 μg butyrylcholine before and D injection of 5 μg butyrylcholine after application of DFP.

the application of DFP (Fig. 4 C), but afterwards the same dose had an enormous effect (Fig. 4 D), lasting for a minute at least. A moderate increase was also observed for propionylcholine and a small one for benzoylcholine. For acetylthiocholine, however, no definite potentiation was found. The effect of lobeline was not affected by the treatment with DFP.

The spontaneous activity during ventilation with oxygen showed no increase after DFP, but if oxygen lack was established, the response was definitely increased by DFP (Cp. LANDGREN, LILJESTRAND and ZOTTERMAN 1952).

3. 302 I.S. and 306 I.S.

Neither 302 I.S. nor 306 I.S., applied in concentrations up to 20 mg per ml gave a clear-cut increase in the sensitivity to acetylcholine or oxygen lack.

Discussion.

The great sensitivity of the chemoceptors of the carotid body to acetylcholine has been confirmed in these experiments. It has also been demonstrated that several other choline esters can

stimulate in minute doses. For acetylthiocholine and butyrylthiocholine the sensitivity seems to be about as great as for acetylcholine, though there are individual variations between different cats in this respect. Propionyl- and butyrylcholine are only slightly less active than acetylcholine and benzoyl-, succinyl- and acetyl-β-methylcholine considerably less active.

It has also been found that local application of the cholinesterase inhibitors mintacol and DFP greatly increases the sensitivity of the carotid body to several choline esters. Thus after suitable treatment with mintacol or DFP 1/10 of the dose of acetylcholine normally necessary to produce an effect has been active; strong potentiation was observed for propionyl-, butyryl- and benzoyl-choline, whereas with acetylthiocholine and butyrylthiocholine the results were different, a decreased response being obtained after mintacol and no alteration after DFP. The effects of 302 I.S. and 306 I.S. were doubtful.

The occurrence of cholinesterases in the carotid body (Hollinshead and Sawyer 1945, Koelle 1950, 1951) and the great sensitivity of the chemoceptors to choline esters make it natural to assume that such esters are concerned in some essential process in the glomus. This is further emphasized by the stimulating action of other synaptotropic substances like nicotine. The fact that the effect of oxygen lack may likewise be enhanced by cholinesterase inhibitors and decreased or abolished by drugs that antagonize acetylcholine (Landgren, Liljestrand and Zotterman 1952) indicates that acetylcholine or related substances are normally involved in the initiation of the stimulation of the chemoceptors. It is true that higher doses of the inhibitors or the antagonizing drugs are often required in order to influence the response to oxygen lack than for choline esters injected directly into the carotid artery. But this is likely to be because the drugs administered into the carotid have better access to the choline esters injected than to such substances formed within the cells of the carotid body.

Hollinshead and Sawyer, however, from their determinations of the cholinesterase activity of the carotid body conclude that the humoral mediator between chemoceptor cell and sensory nerve fibre is not acetylcholine. After driving the blood out of the tissues by perfusion through the heart with physiological saline solution they found the number of mg acetylcholine hydrolyzed by 100 mg tissue per hour to be 28.4 for the superior cervical

ganglion of the cat and 2.33 for the carotid body. The corresponding values for acetyl-β-methylcholine (hydrolyzed by the specific cholinesterase) were 7.98 and 0.48 and for benzoylcholine (hydrolyzed by the non-specific cholinesterase) 7.65 and 0.83, respectively. Unfortunately no correction seems to have been made for accumulation of fluid in the tissues. The richness of vascular supply in the carotid body by comparison with the superior cervical ganglion may be an important source of error. But even if it is admitted that the carotid body has a low cholinesterase activity by comparison with the superior cervical ganglion — and this also seems probable from KOELLE's determinations — it does not follow that choline esters do not act as transmitters for the stimulation of the chemoceptive afferents. It is not unlikely that the type of coupling required is quite different in the two cases.

We had hoped to be able by using cholinesterase inhibitors with specific actions on the different kinds of these enzymes to find out which group is of greatest importance in the physiological stimulation of the chemoceptors. Unfortunately this has not been possible. 302 I.S. and 306 I.S. have been found in vitro to inhibit specifically the true and the pseudocholinesterases, respectively (BOVET-NITTI and BOVET 1953). Both of them, however, are very little active in the carotid body. Recent observations on other tissues (KOELLE 1953) suggest that there are more than two enzymes acting on the choline esters, and this further hampers the analysis at present.

In a recent publication HEYMANS, DELAUNOIS, MARTINI and JANSSEN (1953) report that neostigmine, applied locally on the carotid sinuses and the carotid bodies of the dog, induced a moderate respiratory stimulation and increased the sensitivity of the chemoceptors. Eserine also increased the sensitivity towards acetylcholine and cyanide but did not lead to a greater ventilation. Atropine, applied in the same way, abolished the action of acetylcholine and cyanide on the chemoceptors. Ganglionic blocking drugs, such as tetraethylammonium and hexamethonium, did not significantly affect the respiratory response to acetylcholine or cyanide. The authors conclude that their observations do not support the suggestion that acetylcholine serves as a chemical transmitter within the carotid body. No alternative interpretation of the findings is offered. A close study of the results, however, shows that they are not incompatible with our views.

Thus, the stimulating effect of neostigmine, which confirms

our observation of the increased chemoceptor discharge in the sinus nerve (LANDGREN, LILJESTRAND and ZOTTERMAN 1952), is obviously what is to be expected if choline esters act as transmitters. It was pointed out that this substance in our experiments probably had a stronger effect than eserine; when HEYMAN et al. found no stimulating action on respiration this can easily be explained by the policy adopted: real significance was only attributed to those experimental results, where it was possible to obtain practically 100 per cent differences in response. The danger of using such an arbitrary criterion is obvious. In the cat the greatest increase in ventilation that can be obtained by oxygen lack, *i. e.* by maximal stimulation of the chemoceptors, is only about 100 per cent (v. EULER, LILJESTRAND and ZOTTERMAN 1939).

The results with atropine also agree with our earlier observations. The Belgian group interpret the effect as due to the local anesthetic property of atropine and refer to the publication of DE ELIO (1948). This paper, surprisingly enough, contains the following sentence: "Further investigation may therefore indicate that a local anesthetic is essentially a substance which opposes the action of acetylcholine." Though this question is by no means settled, there seems at any rate no reason to take the atropine results as evidence against cholinergic transmission.

With regard to the action of ganglionic blocking drugs the only curve published by HEYMANS et al. shows a clear reduction of the respiratory response to lobeline, acetylcholine and possibly also to cyanide after local application of hexamethonium. This is in good agreement with earlier findings that with hexamethonium the effect of oxygen lack on the chemical action potentials could be abolished (LANDGREN, LILJESTRAND and ZOTTERMAN 1953, GOLLWITZER-MEIER and WITZLEB 1953), the same was found for tetraethylammonium or decamethonium (LANDGREN, LILJESTRAND and ZOTTERMAN 1952) as well as for pentamethonium (GOLLWITZER-MEIER and WITZLEB).

The experimental data of HEYMANS et al. seem actually to support the view that choline esters act as chemical transmitters within the carotid body.

Summary.

Minute amounts of a number of choline esters, injected into the carotid artery, elicited increased chemoceptor activity of the

sinus nerve. The effect was especially pronounced for acetyl-, acetylthio- and butyrylthiocholine; propionyl- and butyrylcholine were slightly less active, whereas for benzoyl-, acetyl-β-methyl- and succinyl-choline somewhat larger doses were required.

After local treatment of the carotid body with the anticholinesterase mintacol the responses to acetyl-, propionyl-, butyryl- and benzoyl-choline were greatly increased, but the sensitivity to the thiocholines was lowered. Oxygen lack led to a stronger stimulation after mintacol than before.

DFP, applied locally, also led to a sensitization of the chemoceptors to acetyl-, butyryl-, propionyl- and benzoylcholine as well as for oxygen lack. Acetylthiocholine was again an exception.

The results support the view that acetylcholine or related substances are involved in the initiation of the chemoceptive discharge from the carotid body.

References.

Bovet-Nitti, F. and D. Bovet, Arch. exp. Path. Pharmak. 1953. *220*. 52.

De Elio, F. J., Brit. J. Pharmacol. 1948. *3*. 108.

Euler, U. S. v., G. Liljestrand and Y. Zotterman, Upsala Läkarfören. Förhandl. 1939. *45*. 373.

— — — Acta physiol. scand. 1941. *1*. 83.

Gollwitzer-Meier, K. and E. Witzleb, Arch. ges. Physiol. 1953. *256*. 381.

Heymans, C., A. L. Delaunois, L. Martini and P. Janssen, Arch. int. Pharmacodyn. 1953. *96*. 209.

Hollinshead, W. H. and C. H. Sawyer, Amer. J. Physiol. 1945. *144*. 79.

Koelle, G. B., J. Pharmacol. 1950. *100*. 158.

— Ibidem 1951. *103*. 153.

— Biochem. J. 1953. *53*. 217.

Landgren, S., G. Liljestrand and Y. Zotterman, Acta physiol. scand. 1952. *26*. 264.

— — — Arch. exp. Path. Pharmak. 1953. *219*. 185.

— — — Acta physiol. scand. 1954. *30*. 149.

Liljestrand, G., Ibidem 1951. *24*. 225.

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Insulin and Glucose Uptake by the Liver.

By

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The directly observable effect of insulin, the fall in blood glucose concentration, is mainly due to an increase in the rate of glucose transfer from the blood to the tissues. This effect on the glucose transfer must be considered the principal, if not the only effect of insulin (LUNDSGAARD 1939). Whether the glucose entering the tissues under the influence of insulin is by preference oxidized or stored as glycogen shall not be discussed in this paper. When the blood glucose concentration is fairly high, the influx of glucose into the muscle tissue may reach such high rates that the major part of the glucose entering the muscle cells must be stored as glycogen, as the rate of oxidations cannot account for the amount of glucose disappearing.

It is unanimously agreed that insulin enhances the uptake of glucose in muscle tissue. This has been demonstrated repeatedly on various types of muscle preparations: eviscerated animals (BURN and DALE 1926), hind limb preparations (LUNDSGAARD, NIELSEN and ØRSKOV 1939 b), the rat diaphragm (GEMMILL and HAMMAN 1941).

The question whether insulin enhances the glucose uptake in the liver, however, has been answered differently by different investigators. It is well established that administration of insulin to a diabetic organism evokes storage of glycogen in the liver. In contrast, it is equivocal if this effect is produced directly by the action of insulin on the liver tissue or if it is an effect secon-

dary to the normalization of the carbohydrate metabolism of the peripheral tissues and the cessation of the glucose loss through the kidneys. It has never been clearly shown that insulin induces an increase in glucose uptake by the normal liver.

The literature dealing with the effect of insulin on the liver is very comprehensive and rather difficult to evaluate. In most experiments carried out on normal, intact animals administration of insulin has caused a decrease in liver glycogen. This effect is generally thought to be due to a secondary mobilization of adrenaline evoked by the fall in blood sugar concentration to hypoglycemic levels. In some of the few experimental series, in which an increase in liver glycogen was found after administration of insulin, this increase was observed some hours after insulin had been given and might have been caused by a mobilization of lactic acid from the muscles evoked by a secondary mobilization of adrenaline (CORKILL 1930 and RUSSELL 1938).

The demonstration that some insulin preparations contain a glycogenolytic factor (DE DUVE, HERS and BOUCKAERT 1946 and SUTHERLAND and CORI 1948) complicates the evaluation of the somewhat older experiments as one does not know whether the insulin employed has contained this factor or not. The same complication is met with in the evaluation of experiments on isolated livers and on liver slices or liver pulp.

The insulin preparation used by LUNDSGAARD, NIELSEN and ØRSKOV (1939 a) in their experiments on isolated, artificially perfused livers presumably did not contain any glycogenolytic factor. Nevertheless, no effect of insulin on the glucose exchange between blood and liver tissue was demonstrable in these experiments.

SECKEL (1938) reported that insulin inhibits the spontaneous glycogenolysis in livers slices. Such an inhibition of glycogenolysis is of course not the same thing as a promotion of the transfer of glucose from blood to liver tissue. Moreover, LUNDSGAARD, NIELSEN and ØRSKOV (1939 a) in their experiments on isolated rabbit livers were unable by means of insulin to inhibit glycogenolysis evoked by addition of adrenaline.

The strongest evidence that the liver plays an important part in the uptake of glucose evoked by insulin has been presented by DE DUVE, NAYER, OOSTVELDT and BOUCKAERT (1945). These investigators have compared the rate of disappearance of glucose after large ("supramaximal") doses of insulin in normal, hepatectomized and eviscerated dogs. The rate of glucose uptake in the

tissues was determined as the rate of glucose infusion necessary to keep the blood sugar level constant. A correction was introduced for the unavoidable minor changes in the blood glucose concentration from the beginning to the end of the experimental period. The authors state the glucose uptake in g/kilo/80 minutes. Expressed in this way the uptake at normal blood sugar concentration (87 mg%) was for the three groups of experiments: normal dogs 2.10, hepatectomized dogs 0.40 and eviscerated dogs 0.40.

According to these figures the liver should play a predominant part in the uptake of glucose after the administration of large doses of insulin to normal dogs. In a dog with a body weight of 20 kilos and with a liver weighing about 500 g the liver should take up 34 g of glucose during the experimental period of 80 minutes. This is a very large amount, but it does not exceed what might be possible.

I have repeated the experiments of the Belgian investigators on cats. The reason why cats were chosen was mainly, that most of the experiments in isolated, artificially perfused liver performed in my laboratory have been performed on cat livers. In the isolated cat liver no net uptake of glucose is observed and no such net uptake can be induced by insulin. Since it is often claimed that this might be due to some impairment of the liver caused by the perfusion technique, it was thought worth while to see if it is possible to demonstrate an effect of insulin on the glucose uptake in the cat liver *in situ* by using the approach of the Belgian investigators.

Technique.

The cats were anaesthetized with amytal. The hepatectomy was carried out with a technique closely resembling the technique used by the Belgian investigators. The peripheral end of the stem of the portal vein was connected with the right renal vein by means of a cannula. Unfortunately the right renal vein is often double and the veins are in that case generally too small to serve as outflow for the portal vein. If hepatectomy could not be carried through, the animal was eviscerated and used for the experiment in that state. Clotting was prevented by heparin.

The body weight of the cats has varied slightly around 3 kilos. Insulin ("Novo", 40 units per 3 kilos body weight) was injected intravenously.

Glucose was injected at a constant rate from a motor driven syringe. The volume injected per unit of time was kept constant from ex-

periment to experiment but the concentration of the glucose solution was varied. By this variation in the amount of glucose injected per unit of time, and by varying the interval between hepatectomy or evisceration and the start of the experimental period the average blood sugar concentration during the experimental period was made to vary rather considerably. The blood glucose concentration was determined by the Hagedorn-Jensen method.

The correction for changes in the blood sugar concentration from the start to the end of the experimental period was based on the supposition that 20 % of the body weight acts as solvent for free glucose to the same extent as does whole blood. As the changes in blood sugar concentration in the experimental periods have been only small the correction has influenced the results only slightly.

To facilitate a direct comparison with the results of the Belgian investigators I have used the same experimental period of 80 minutes and have expressed the uptake of glucose by the tissues in g/kilo/80 min.

Results and Conclusions.

As will be seen from fig. 1, no difference in the rate of glucose uptake by the tissues was found among the three groups of experiments. In the cat the liver apparently takes no part in the uptake of glucose by the tissues under the influence of insulin.

At blood sugar levels about 90 mg% the uptake of glucose in normal as well as in hepatectomized and eviscerated cats was of the same order of magnitude (about 0.40 g/kilo/80 min.) as the uptake found by the Belgian investigators in hepatectomized and eviscerated dogs. No conclusions, however, ought to be based on this coincidence.

In a series of experiments on eviscerated cats to which no insulin was given, a glucose uptake by the peripheral tissues of about 0.15 g/kilo/80 min. was found at an average blood glucose concentration of 114 mg%. When this finding is compared with the results presented in fig. 1, it is seen that a large dose of insulin at this blood sugar level triples the uptake of glucose by the peripheral tissues in cats.

Within the rather narrow blood glucose concentration range within which the Belgian investigators have worked the variations of the glucose uptake with the blood sugar concentration was found to be rectilinear. The results of my experiments, which have been carried out over a somewhat wider glucose concentration range, show that the increase in glucose uptake with increasing glucose concentration tends to level off at the higher glucose

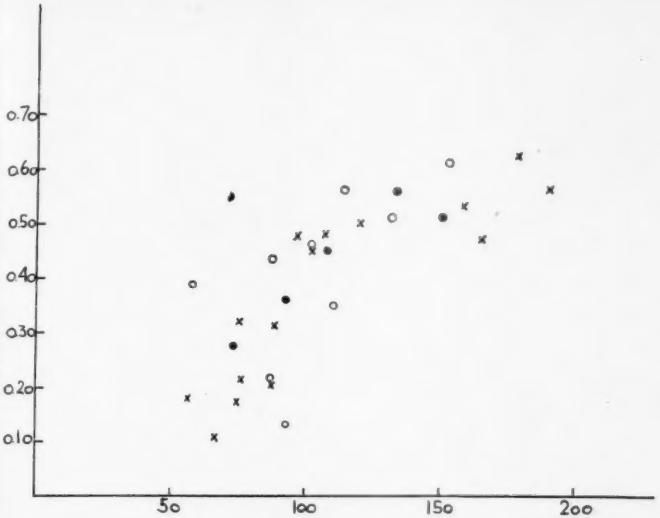

Fig. 1. Glucose uptake by the tissues after a large dose of insulin.
Ordinates: Glucose uptake in g/kilo/80 min.
Abscissas: Blood glucose concentration in mg%.
×: experiments on normal cats. ○: experiments on hepatectomized cats.
●: experiments on eviscerated cats.

concentrations. The form of the curve which might be drawn in fig. 1 thus resembles the curve found by WIERZUCHOWSKI (1936) in normal dogs.

Summary.

The rate of glucose uptake by the tissues after a large insulin dose has been determined in normal, hepatectomized and eviscerated cats. No difference in the rate of uptake, or in the dependence of the rate of uptake on the glucose concentration, was found among the three groups of experiments.

Apparently in the cat the liver takes no part in the glucose uptake by the tissues under the influence of insulin.

The inability to induce a net glucose uptake in the isolated cat liver by means of insulin is consequently scarcely due to an impairment of the liver caused by the perfusion technique.

References.

Burn, J. H. and H. H. Dale, J. Physiol. 1924. *59*. 164.
Corkill, B., Biochem. J. 1930. *24*. 779.
Duve, Chr. de, P. P. de Nayer, M. van Oostveldt and J. P. Bouckaert, Arch. int. Pharmacodyn 1945. *70*. 78.
— H. G. Hers and J. P. Bouckaert, Ibidem 1946. *72*. 45.
Gemmill, D. L. and L. Hamman jr., Johns Hopk. Hosp. Bull. 1941. *68*. 50.
Lundsgaard, E., Uppsala Läkareför. Förh. 1939. *45*. 143.
— N. A. Nielsen and S. L. Ørskov, Skand. Arch. Physiol. 1939. *81*. 11.
— — — Ibidem 1939. *81*. 20.
Russell, J. A., Amer. J. Physiol. 1938. *124*. 774.
Seckel, H. P. G., Endocrinology 1938. *23*. 760.
Sutherland, E. W. and C. F. Cori, J. Biol. Chem. 1948. *172*. 737.
Wierzuchowski, M., J. Physiol. 1936. *87*. 311.

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The Potassium Absorption by Pigeon Blood Cells.

A Considerable Potassium Absorption by Pigeon- and Hen Blood Cells is Observed when a Hypertonic Sodium Chloride Solution is Added.

By

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Received 4 March 1954.

In a former paper (ØRSKOV 1948) it was shown that bacillus coli communis in a hypertonic solution is able to absorb potassium, and the plasmolysis will then disappear. This process is very fast so that it will be finished after a few minutes, and during this time the cell potassium may have increased by 30 to 70 %. Of course it was very interesting to find out whether such processes did also take place in other kinds of cells. With yeast cells I have not been able to find a similar behaviour, but already in 1947 I made some experiments with blood from pigeons, and got results which were to a certain extent similar. Blood cells from birds are rather difficult to handle, they will easily haemolyze, and the haemoglobin is more sensitive than haemoglobin from mammals. Another thing appeared very soon, namely that the blood cells from pigeons would absorb potassium in a rather high degree even if nothing was added to the blood (tables I and II). It was shown when the blood was defibrinated, and also when heparin had been added to the blood. What is the cause of this potassium absorption I have not been able to find out. The blood is drawn in a very simple way. The neck of the pigeon is cut through with a pair of scissors, and I have thought that addition of tissue fluid might be the cause of the changing of the blood cells. I have tried to draw the blood with a syringe from a vein,

but this is rather difficult, and no difference in the results was observed. Then I have considered the possibility of substances given off from the glass, and have carried out a very thorough cleansing of the glasses, and in some experiments the glasses were silicone-treated, but no change was observed. A lot of causes may be thought of to produce this potassium absorption, but I have not been able to approach the problem.

Methods.

The way of bleeding the birds has already been mentioned. After several experiments it was found that the most appropriate way to deal with the blood is to have it in an Erlenmeyer flask of 50 ml, close it with a rubber stopper, and place it in a water bath of 43°. The flask is rocked from side to side about 45 times a minute. The distance from the axis to the blood surface is 17 cm, and the angle of the movements 25°. The water in the bath must almost reach the top of the flask. Potassium and sodium determinations are made with Beckman's flame photometer after the blood cells and the plasma have been electrolyzed (Ørskov 1952). Chloride determinations are made by electrometric titration after precipitation of the plasma proteins and cell proteins with trichloro-acetic acid. Haematocrit determinations are made with the haematocrit tubes used in this institute (Ørskov 1945). The blood is centrifuged at 10,000 rounds a minute for ten minutes.

Experiments.

One of the first problems which had to be investigated was whether oxygen was necessary for the absorption of potassium. It has been shown for liver cells (Flink, Hastings and Lanry 1950), coli bacilli (Ørskov 1948), and also for yeast cells (Conway and O'Malley 1945). With blood from pigeons I have in some instances been able to show the importance of oxygen, but in other experiments it has not been possible.

In table I I am giving an example where I didn't succeed. Centrifuge glasses (10 ml) were filled with blood and closed with a rubber stopper, and allowed to stand at 43° for different times. After these times half of the blood has been taken as controls, and the rest has been shaken with oxygen to show if a potassium absorption will now take place. It is quite evident from the table that no such potassium absorption has taken place.

In quite similar experiments (table II) the blood is allowed to stand in the glasses for 3 hours, then a sample is taken, and this

Table I.

Blood in heparin from 3 pigeons is mixed. 4 centrifuge glasses (10 ml) are filled with blood and closed (no air above). Water bath 43°.

Time in minutes	Haematocrit values	Plasma K mM/l	Plasma Na mM/l	Rest of the blood aerated with O_2 and left in bath for 30 minutes		
				Haematocrit	Plasma K mM/l	Plasma Na mM/l
0	55	6.12	149			
60	57	2.63	154	55.5	5.23	154
120	60.5	6.96	157	59	6.97	158
160	61.5	10.47	157	60	11.32	160
200	63	12.55	159	61.5	14.29	160
No haemolysis.				After oxygenating about 1 % haemolysis.		

Table II.

Blood in heparin from 2 pigeons.

Time in minutes	Haematocrit values	Plasma potassium mM/l	Plasma sodium mM/l	Haemolysis in %
0.....	57	5.50	142	0
2 centrifuge glasses (10 ml) are filled with blood and closed (no air above). Water bath 43°.				
180.....	67	14.63	150	0
Part of the blood is rocked in a 25 ml Erlenmeyer flask at 43° (atmospheric air).				
240.....	66.5	10.0	157	0
The rest of the blood, 9.1 g is added 0.46 ml 1,050 mM/l NaCl and rocked at 43° for one hour in a 25 ml Erlenmeyer flask (atmospheric air).				
180.....	53	10.1	214	0
240.....	52	7.49	214	0

time it is possible to show that the rest after having been shaken with air and rocked for 1 hour is able to absorb potassium. In another portion of the blood sodium chloride was added, and here there evidently was an absorption of potassium.

In table III you see an experiment where the blood is aerated with carbon dioxide for ten minutes. A sample is taken, and the rest of the blood is allowed to stand at 43°, and samples are taken at different times. It is seen from table III that the aerating has caused a certain haemolysis, and the loss of potassium is rather considerable after 100 minutes.

Now the blood is aerated with oxygen, and potassium absorption takes place at high rate, and after 90 minutes 10 mM/l of the plasma

Table III.

Time in minutes	Haematocrit values	Plasma potassium mM/l	Plasma sodium mM/l	Haemolysis in %
0.....	52	4.22	168	0
The blood from 3 pigeons is aerated with 100 % CO_2 in a 200 ml flask for 10 minutes. The samples are taken without admission of atmospheric air.				
10.....	57	8.15	180	2
30.....	58	9.88	180	3
55.....	59	13.00	186	4
100.....	61	17.8	200	6
The blood is aerated with oxygen and rocked.				
10.....	56	21.2	177	12
20.....	56.5	18.7	180	15
45.....	57	13.74	180	16
90.....	57	10.78	192	18

potassium have been absorbed. The absorption in this experiment is in reality much greater as the haemolysis still goes on, and in the last sample it amounted to 18 % of the blood cells of the blood. From the haematocrit values it is seen that the blood cell volume is increasing, and after the blood has been aerated with oxygen the volume has fallen to a considerable extent. The question may be put: what is the reason that potassium absorption cannot be shown in all experiments? Perhaps the long time of lack of oxygen has had an injurious effect on the blood cells. But it may also be imagined that some substances present in the blood when taken from the pigeons have disappeared. If you draw blood from a hen or a cock, and keep it at body temperature, you will find that the potassium concentration of the plasma is constant for hours when the blood is rocked in the way previously described. The same is the case if you draw blood from man and rabbit, though here a slight degree of potassium absorption can be seen. If you augment the osmotic pressure with a substance which is not able to penetrate the blood cell membrane, you will find that the blood cells from pigeons as well as hens are able to absorb potassium, and under certain circumstances in a very high degree.

This is seen in table IV, in which 3 experiments are stated.

1. *No addition.* Here the potassium concentration during 45 minutes decreases with about 2.5 mM/l, after 90 minutes the original concentration is reached, after 180 minutes an increase is found.

Table IV.

Each of the 3 experiments is made with blood added heparin from a pigeon. Water bath 43°. 50 ml Erlenmeyer flask, rocked.

Time in minutes	Haematocrit values	Plasma potassium mM/l	Plasma sodium mM/l		Haemolysis in %
0....	53	5.75	141		0
45....	54	3.16	148		0
90....	52.8	5.87	148		0
180....	52.8	8.70	146		0
The blood is added 1/20 of the volume of a 175 mM/l KCl solution.					
0....	50	23.0	129		0
45....	51	19.7	139		0
90....	50	22.4	136		0
180....	49	24.6	133		0
The blood is added 1/20 of the volume of a solution containing 350 mM/l KCl and 1050 mM/l NaCl.					
				Cell Chloride mM/l	
0....	41	32.9	206	70	0
45....	42	28.2			0
90....	43	23.0	222	90	0
180....	45.5	15.0	237	97.5	0

2. The KCl concentration is much higher, but the changes are nearly the same as in 1.

3. NaCl and KCl have been added, and a very pronounced KCl absorption has taken place. If the original KCl concentration of the red cells is estimated to 105 mM/l, and the haematocrit to 52.3 an increase in the cell potassium of 20.5 % after 3 hours has taken place. The corresponding chloride concentration has increased 56 %.

In table V two corresponding experiments are made with blood from a cock. In one part of the blood nothing has been added, and here the potassium concentration of the plasma is unchanged during the experiment. To the other part of the blood sodium chloride and potassium chloride have been added, and here a marked potassium absorption has taken place.

If you make corresponding experiments with pigeon blood at 20° you will find that the potassium absorption still goes on though at a much slower rate, but with blood from a hen you will see that nothing happens even if you add sodium chloride, so that the osmotic pressure has been augmented.

Table V.

Blood from a cock is added heparin. The blood is distributed in two 50 ml Erlenmeyer flasks, rocked at 42°.

Time in minutes	Haematocrit values	Plasma potassium mM/l	Plasma sodium mM/l.	Haemolysis in %
		Nothing added.		
0.....	51	6.36	152	0
20.....	50.5	6.37	155	1
45.....	50.3	6.18	155	1
90.....	50.5	6.14	155	1
180.....	49.5	8.52	155	2
1/20 volume of a solution containing 175 mM/l KCl and 1,050 mM/l NaCl is added.				
0.....	40	20.0	208	0
20.....	41.5	19.5	213	1
45.....	42	17.7	213	1
90.....	42	15.2	211	1
180.....	43	12.14	201	2

Discussion.

The most valuable information the abovementioned experiments have given is that blood cells from fowls are very suitable for permeability experiments. I don't think that any tissue from an animal used for such experiments has formerly been able to give a corresponding degree of potassium absorption, and especially it must be borne in mind that the blood cells are quite normal. It has formerly been shown that when blood cells from mammals have been stored for a long time at 4° at which time they have lost a lot of potassium and taken up a corresponding amount of sodium, they are able to extrude the sodium, and once more take up the potassium, when the blood is placed at a higher temperature.

This most important observation was made by HARRIS (1941). MAIZLES (1952) has recently shown that chicken blood behaves in the same way after 7 days at 4°. He was able to show that oxidation was important for a later absorption of potassium as this process could be prevented by addition of cyanide.

But with blood which has been newly drawn such changes have not been shown. Quite another thing is how these experiments are to be explained. In experiments with coli bacilli I cautiously said that the cells had a regulation so that when they were plasmolysed by a hyperosmotic pressure they would absorb potassium so that the plasmolysis disappeared, and it could be shown that

Table VI.

Water bath 43°. Rocked in a 50 ml Erlenmeyer flask. Blood from a pigeon is added 1/40 volume of a solution containing 40 % ethyl alcohol and 0.9 % NaCl.

Time in minutes	Haematocrit values	Plasma potassium mM/l	Plasma sodium mM/l	Haemolysis in %
0.....	52	4.77	144	0
10.....	52	5.60	148	0
20.....	52	5.50	147	0
30.....	52	6.12	148	0
40.....	52	7.46	148	1
50.....	51	9.55	143	1
Blood from a pigeon is added 1/20 volume of a solution containing 20 % ethyl alcohol, 1050 mM/l NaCl and 350 mM/l KCl.				
Before addition.				
0.....	52	5.53	149	0
After addition				
0.....	41	31.3	202	0
10.....	41	29.5	197	0
20.....	41.5	29.5	203	0
30.....	42	29.5	201	0
40.....	42	27.5	202	0
90.....	43	22.4	202	0
180.....	45.3	16.1	200	0

also other substances were able to abolish the plasmolysis. But of course this was no explanation. I realize now that there are two possibilities of explaining the procedure. 1) The active process of absorbing potassium can be thought to be augmented when the osmotic pressure is increased. 2) And the other possibility is that the loss of potassium through the membrane of the blood cells is lessened. A final proof of which of these processes is the most important can no doubt be shown by using radiactive potassium. When I have not at this time made such experiments it is because I know that blood from fowls is very easily damaged so that the results may be difficult to interpret. I think that the most probable explanation of the above experiments is that the membranes of the blood cells are tightened by the hypertonic pressure of the plasma.

In table VI I have two experiments where alcohol has been added to the blood. In one experiment where nothing else has been added to the blood you see that potassium is lost from the blood cells, but if you also add hypertonic sodium chloride solution to the blood you see that potassium is absorbed at a high rate from the

plasma. I think it is rather probable that alcohol has caused a greater permeability of the blood cell membrane, and that the membrane by adding sodium chloride is again tightened, and the loss of potassium through the membrane has ceased, or at any rate been lessened to a great extent. At present I think that my experiments indicate that there are two active processes, 1) an active potassium absorption, and 2) an active sodium extrusion, and then there is 3) an interchange of potassium and sodium from the outside and the inside of the membrane, and finally 4) that potassium can pass out through the membrane together with chloride and bicarbonate ions. The last two processes may be hampered by hyperosmotic pressure. In most of the tables sodium movements are also seen, but in many cases they do not correspond with the potassium movements. It is to be mentioned that the membrane of pigeon blood cells does not behave in the same way at a hypertonic pressure as for instance the membrane of human blood cells. Here the membrane is highly crenated, but with pigeon blood cells the surface appears to be quite smooth. At very high hyperosmotic pressures you can see that the blood cells have become more flat, and that the nucleus is more prominent. It is quite probable that such a change of shape will make the membrane less permeable, but still it is possible that the apparatus of active absorption of potassium and extrusion of sodium will not be changed by the process.

From recent papers on the permeability of mammalian blood cells it appears that an active potassium absorption, and an active sodium extrusion is considered to be a probable explanation.

In this connection papers of PONDER (1950), SOLOMON (1952), and HARRIS and MAIZELS (1952) can be mentioned.

It has not been mentioned before that in Table IV, V and VI the potassium absorption is accompanied by a distinct rise of the haematocrit values which was also to be expected.

Summary.

It is shown that freshly drawn pigeon blood when placed at body temperature during the first hour will absorb half or more of the plasma potassium. Later the plasma potassium concentration will increase. Lack of oxygen will stop the potassium absorption, and instead potassium of the cells is lost, and sodium increased.

Addition of isotonic KCl solution will not change the potassium absorption.

But if you besides KCl add a hypertonic solution of NaCl a considerable potassium uptake is seen, and it will carry on for more than three hours. In this way the cell potassium concentration can be augmented by more than 20 %.

Blood from hen or cock will not absorb potassium unless a hypertonic NaCl solution is added.

The hypertonic solution may increase the active potassium absorption or it may tighten the cell membrane so that the loss of potassium from the cells is decreased. The second possibility is considered the most probable. The theory is strengthened by an experiment where 1 % ethyl alcohol produced a loss of potassium, whereas simultaneous addition of hypertonic NaCl solution produced a considerable potassium uptake. Simultaneously with the potassium absorption an increase of the haematocrit values is seen.

The final solution of the problem may be given by experiments with radioactive potassium.

The experiments correspond to similar experiments made with bacillus coli communis (ØRSKOV 1948).

References.

CONWAY, E. J. and E. O'MALLEY, Biochem. J. 1946. *40*. 59.

FLINK, E. B., A. BAIRD HASTINGS and J. K. LANRY, Amer. J. Physiol. 1950. *163*. 598.

HARRIS, J. E., J. Biol. Chem. 1941. *141*. 579.

— and M. MAIZELS, J. Physiol. 1952. *118*. 40.

MAIZELS, M., Ibidem 1952. *118*. Proceed. 36 P.

ØRSKOV, S. L., Acta path. microbiol. Scand. 1945. *22*. 523.

— Ibidem 1948. *25*. 277.

— Acta Physiol. Scand. 1952. *27*. 321.

PONDER, E., J. gen. Physiol. 1950. *33*. 745.

SOLOMON, A. K., Ibidem 1952. *36*. 57.

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The Effect of Increasing Concentrations of Tween 20 (Polyoxyalkylene Sorbitan Laurate) on the Surface Tension of Plasma.

By

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When investigating the effects of some surface active agents on various physicochemical characteristics of plasma, it was considered important first to study the effect of surface active agents on the surface tension of plasma. It has been claimed that there is in blood or in the organism as whole a regulatory mechanism tending to preserve the surface tension at an approximately constant value (BRINKMAN 1923, NEUBAUER 1924, ADLER 1925, ADLERSBERG and SINGER 1925, DU NOÜY 1926, KAUNITZ and KENT 1936, GIGON and NOVERRAZ 1939). These studies have been made using lecithin, bile salts or sodium oleate. The present paper is a report of a study of the effects of various concentrations of Tween 20 on the surface tension of human plasma in vitro. This is a non-ionic surface active agent and consequently does not as known from the studies of LUNDGREN (1945) and GLASSMAN (1948) combine with proteins.

Material and Methods.

The surface tension was measured with the DU NOÜY Tensiometer (Cambridge Instrument Company, Ltd., 13. Grosvenor Place, London, S. W. 1.). The temperature of the solution to be measured was recorded with a thermoelectric thermometer (Thermorapid Medeor G. m. b. H. Hamburg 20). The surface-active agent employed, Tween

20 (Georg T. Gurr Ltd., London, S. W. 6.), is a non-ionic surface active agent, polyoxyalkylene sorbitan laurate.

The platinum ring, the platinum wire and the glassware were cleaned by soaking them first in 5 per cent NaOH-solution, and then in cleaning mixture (a moderately strong solution of dicromate and strong sulphuric acid) and finally they were rinsed with liberal amounts of water. The hypodermic needles were cleaned mechanically, with soap solution, and finally with plenty of water.

For each determination, approximately 11 ml. of blood was collected from the cubital veins directly into a centrifuge tube, (to which 2 drops of heparin Liquemin, Hoffman-La Roche had been placed to prevent coagulation). The blood was centrifuged immediately, 5 ml. of plasma was pipetted on a watch glass, and the measurements were carried out immediately.

Because it has been observed that even small impurities may affect the surface tension, the cleanliness of the equipment was checked every time by measuring the surface tension of water brought in contact with all the requisites used, *i. e.* needles, centrifuge tube, pipette, watch glass, platinum loop and platinum wire.

The surface tension-concentration curve of each sample was determined as follows: Since it is well known that the agitation of the fluid to be studied, whether it has been long at rest or recently stirred, and on the other hand the time, during which the surface active agent has been in the solution, both affect the surface tension of the solution (*e. g.* SCHWARTZ and PERRY 1949), an attempt was made to standardize these two factors as far as possible in every determination. The platinum ring was lowered into the solution, in which it was left for 1 min. Then it was lifted from the solution, the zero point was checked, and 20 sec. after lifting it was again lowered into the solution, the surface tension was measured immediately, which took about 20—25 sec., the zero point was checked, the ring again lowered into the solution, and the measurement was repeated. Immediately afterwards the platinum ring was again lowered into the solution, the amount of Tween 20 required for the lowest concentration was added, and the solution was thoroughly stirred. After having stirred the solution it was allowed to calm down during 1 min., after which a duplicate determination was carried out as described above, and the amount of Tween 20 required for the next concentration was added, and so on. All measurements were performed in duplicate, and the mean of the duplicate determinations was taken as the value of the surface tension.

The surface tension was measured in this way at the following concentrations of Tween 20: 0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.27, 0.91, 1.80, 3.54, 5.22 and 8.40 per cent. For the concentration range from 0.01 to 0.91 per cent, Tween 20 was used in a 10 per cent aqueous solution (it was observed that the small volume of water added did not affect the surface tension of plasma) for higher concentration undiluted Tween 20 was used.

In order to assess the accuracy of the method, the methodical error

(s) of a determination was calculated with the aid of the formula: $s = \sqrt{\frac{\Sigma \triangle^2}{2\ n}}$ (where $\triangle$ = the difference of duplicate determinations, n = the number of duplicate determinations, in this case 209). This calculation gave the value 0.219 dyne/cm as the methodical error of a single determination, *i. e.* the error due to the act of measurement itself in determining the surface tension of plasma. On the other hand, the results of the measurements of the surface tension of water, made as controls, give some idea of the reliability of the method as a whole, bringing forth the effects of eventual impurities and other uncontrolled factors affecting the results. The real mean value of the surface tension of water is within the limits ($M \pm 3\ \Sigma$, where Σ is the standard error of the mean) 71.194 ± 0.091 dyne/cm.

A temperature correction was not used, because the temperature did not vary essentially.

For comparison, the effect of Tween 20 on the surface tension of water was studied with the same technique.

Thirteen samples of plasma were examined, which were obtained from healthy males whose age varied from 19 to 27 years. The majority of the subjects had been fasting for more than 12 hours, but some had taken a light meal with tea or coffee from 3 to 4 hrs. before taking the blood sample. Ten surface tension-concentration curves were made on water.

Results.

The results are shown in the figure, in which the ordinate represents the surface tension and the abscissa the concentration of Tween 20. The figure shows two zones, zone A (hatching) which covers the surface tension-concentration curves of plasma, and zone B covering those of water. It is observed that the surface tension-concentration curve of plasma falls less steeply than that of water, but at high concentrations of Tween 20 both curves meet.

Discussion.

The results show that the surface tension of plasma falls less than that of water at low and medium concentrations of Tween 20. Plasma appears to contain some kind of "buffer" which reduces the action on surface tension of the surface active agent examined. Because the surface tension is proportional to the concentration of the surface active agent on the interface, it is evident that in plasma a portion of the surface active molecules lose their surface activity in this sense, either through structural

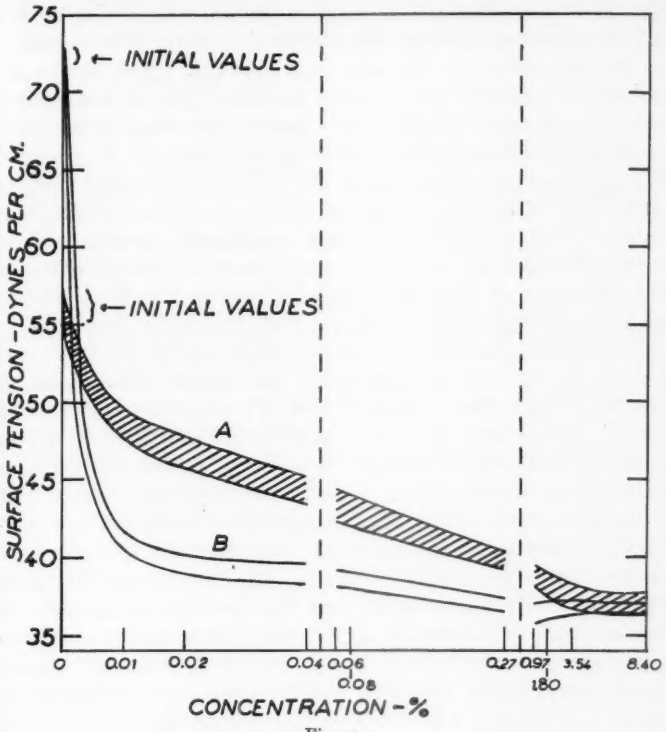

Figure.

changes or through binding in the interior of the solution. This view is supported also by the observation that at high concentrations the surface tension-concentration curves of plasma and water meet.

BRINKMAN, who by using lecithin observed that blood had some kind of mechanism regulating its surface tension, considers that the phenomenon is at least partly caused by adsorption of lecithin on the erythrocytes. NEUBAUER has studied this phenomenon mainly *in vivo*, observing, however, it also *in vitro*. He considers that the regulation of the surface tension depends on the functions of the organs, mainly of liver and kidney, but he mentions also some kinds of "micelles" secreted by the liver, which may adsorb the bile salts used in his studies. ADLER has

also used bile salts, and he ascribes the reducing action on the fall of the surface tension, excerted in his experiments by serum, to the adsorption of bile salts to cholesterol. These studies by ADLER were criticized by KAUNITZ and KENT, who do not entirely negate a regulation of the surface tension, but consider it, essentially in agreement with NEUBAUER, as being due to parts of the organism other than blood. DU NOÜY has observed that on addition of bile salts or sodium oleate to serum, the surface tension which promptly falls quite considerably returns in 7 or 8 minutes back to its normal level; he ascribed this effect to the adsorption of the surface active agents used on large protein molecules. RUBINSTEIN (1938), on the other hand, has come to the conclusion that the phenomenon observed by DU NOÜY has nothing to do with proteins and is no colloidal phenomenon at all; according to his studies it is due to a reaction of the surface active agents with calcium. GIGON and NOVERRAZ, who also used bile salts, have similarly observed in serum a factor preventing the fall of surface tension, and they, too, ascribe this to an adsorption of bile salts to proteins. Among the new synthetic surface active agents, it has been observed that the ionic surface active agents react with proteins (PUTNAM and NEURATH 1944 and 1945, VALKO 1946), but the non-ionic compounds do not combine with proteins, as has been demonstrated with precipitation (GLASSMAN) and electrophoresis (LUNDGREN) experiments. Accordingly, Tween 20 which is a non-ionic surface active agent, is evidently not bound to the plasma proteins. Plasma, however, being a somewhat inhomogeneous system and containing dissolved surface active agents, provokes an aggregation of surface active molecules to micelles (cf. FISCHER and GANS 1946), which may well explain the flattening of the surface tension-concentration curve demonstrated in this study.

Summary.

The action of various concentrations of the non-ionic surface active agent, Tween 20, on the surface tension of plasma was studied. It was observed that plasma contains a factor tending to suppress the action of the surface active agents on the surface tension. The possible causes of the phenomenon are discussed.

References.

ADLER, A., Z. ges. exp. Med. 1925. *46*. 371.
ADLERSBERG, D. and E. SINGER, Ibidem 1925. *46*. 500.
BRINKMAN, R., Ber. ges. Physiol. 1923. *20*. 312.
DU NOÜY, P. L., Surface Equilibria of Biological and Organic Colloids. New York, The Chemical Catalogy Inc. 1926.
FISCHER, E. K. and D. M. GANS, Ann. N. Y. Acad. Sci. 1946. *46*. 371.
GIGON, A. and M. NOVERRAZ, Helv. med. acta 1939. *6*. 264.
GLASSMAN, H. N., Bact. Rev. 1948. *12*. 105.
KAUNITZ, H. and H. KENT, Z. klin. Med. 1936—37. *131*. 285.
LUNDGREN, H. P., Textile Res. J. 1945. *15*. 335.
NEUBAUER, E., Arch. exp. Path. Pharmak. 1924. *102*. 332.
PUTNAM, F. W. and H. NEURATH, J. Amer. Chem. Soc. 1944. *66*. 692. — J. Biol. Chem. 1945. *159*. 195.
RUBINSTEIN, D., Nature 1938. *142*. 1158.
SCHWARTZ, A. M. and J. W. PERRY, Surface Active Agents. Interscience Publishers, Inc. 1949.
VALKO, E. I., Ann. N. Y. Acad. Sci. 1946. *46*. 451.

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The Assay of Thyroid Preparations Based on Increased Sensitivity to Adrenaline.

Studies on a New Method.

By

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In recent years the chemical structure of a number of hormones has been established and it has become possible to produce them synthetically. This has facilitated their clinical use; variation in hormonal efficacy due to the biological methods of standardization has been eliminated by means of accurate methods of weighing etc. Yet some of the hormones are still tied up with biological standardization either because of the failure, up to date, of producing these hormones synthetically, or because for some reason hormones obtained from the animal world are found more serviceable.

The chemical structure and synthesis of the thyroid hormone, thyroxine, has been known for about 30 years (KENDALL 1919, HARINGTON and BARGER 1927), but in clinical work preparations from the animal world are still in almost exclusive use. This is partly due to the fact that the effect of thyroxine when taken by mouth is weaker than that of desiccated gland and varies considerably in different persons, partly to its comparatively high price. On the other hand it is difficult to use it continuously in injections, which would equalize its effect, because the management of the deficiency of this hormone often requires years of thyroid therapy or may take up the lifetime of the patient.

Biological assay of thyroid preparations is an essential condition for successful clinical treatment. In the meanwhile thyroid

preparations have been difficult to standardize and the percentage of errors involved in the different procedures has been considerable, varying up to 200—300 per cent. The best evidence of the inaccuracy of the methods seems to be the co-existence of at least a score of different methods, of which a number may be in use in the same country. In some countries determination of the biological efficacy of thyroid preparations has been abandoned, and only the total iodine content in the gland is determined; this is the case in the U. S. Pharmacopoeia. Yet it has been proved that the biological efficacy of preparations with even an identical iodine content can show multiple variation (Scandinavian Standardization Commission — Nordiska Specialitetskommissionen 1932), evidently because of the different distribution of the iodine content in the gland between the biologically active thyroxine (thyreoglobulin) and the inactive diiodotyrosine. A chemical determination can thus not be considered sufficient alone.

The methods of standardization have, until now, been based on most variable reactions produced by the thyroid hormone in the organism (Bomskow 1937). Among the most common are Hunt's method (1905, 1925) founded on the increase in resistance to acetonitrile of the mouse, caused by the thyroid hormone, that of Mörch (1928) based on increase in carbon dioxide output and the method of Smith et al. (1947) based on increased sensitivity to oxygen deficiency.

It will apparently be difficult to essentially improve the results obtained by the earlier methods by only reforming methodics, for the possibilities they afford have been very carefully studied. One of the present authors (Peltola 1950) made an effort earlier to find out whether more reliable results could be achieved by some new method based on a reaction caused by the thyroid hormone. The effect of the thyroid hormone in increasing sensitivity to adrenaline was the one then decided upon. For this purpose the dependence of the lethal adrenaline dose (*i. e.* the dose causing 50 per cent mortality (LD50) on the effect of the thyroid hormone was studied. The dependence of this reaction on external factors such as the season, light, sex of the animals, food, temperature etc. was likewise studied. The studies showed that the thyroid hormone occasioned a reduction of the LD50 of adrenaline down to the level of 1/7 of the habitual dose. The effect, which is in direct proportion to the size of the thyroid dose administered, is brought out relatively slowly, reaches its peak after 12 days, and remains on

the same level irrespective of whether and how long the intake of thyroid is continued. The reduction is to some extent, as in other tests, dependent on the temperature of the environment and the light, obviously due to the activation or inactivation of the thyroid of the animals themselves. On the other hand it is practically independent of season, the weight of the test animals, sex and food. In the studies the thyroid hormone was given by mouth, which is in accordance with conditions in clinical administration of the hormone. The adrenaline was injected subcutaneously, which reduced the possibility of technical errors to a minimum. Since the results are read from the number of the dead animals, the possibility of subjective interpretations is also ruled out. The method thus possesses many advantages compared with many other methods of standardization used at present.

In the earlier study, one single thyroid preparation was used. To find out whether the reaction is sensitive enough and whether it follows the same trend when preparations with a different biological efficacy are employed, another research seemed necessary. In this study three thyroid preparations, whose iodine content is determined and biological efficacy would be checked by this and several other methods of standardization and compared with their effect on the human organism.

Methods.

The three thyroid preparations used were in the form of dried powder. No. 1 was a product of the Danish manufacturers A/S Medicinalco, Copenhagen[1] which in Denmark has been used for thyroid preparations. No. 2 was a commercial American product, likewise employed for thyroid preparations in Finland, and No. 3 a preparation made by Orion OY, Pharmaceutical Manufacturers from the thyroids of cattle, collected in Finland. On each brand the total iodine was determined chemically according to the U. S. Pharmacopoeia (USP XIV).

The other biological standardization methods used were: the acetonitrile test and tests based on increase of carbon dioxide output and increased sensitivity to oxygen deficiency. The preparations were compared, further, by using them on patients with hypothyreosis who were under continuous treatment with thyroid preparations.

The acetonitrile test was performed by the Grab method (1932) in the following way: On three successive days the test animals were given, by mouth, thyroid powder in doses which differed from each other by 30 per cent, *i. e.* in 2, 3 and 4.5 per cent solutions; each solution

[1] Our thanks are due to Mr. ERIK JACOBSEN, M. D., head of the scientific laboratories of A/S Medicinalco for sending the preparation.

was administered to 6 mice. Acetonitrile was injected intravenously in doses which were in the ratio 3/4, 4/4 and 5/4 to the lethal dose of control animals. The results of the tests were estimated in Bomskow's (1938) manner.

The determination on the basis of the carbon dioxide output was made by MÖRCH's (1928) method: Male mice weighing 16—22 g, which had been kept on the standard diet for 8 days before the beginning of the test, were used. After this the basal metabolism was registered on 4 successive days, the carbon dioxide output was calculated at + 23° C according to Haldane's open principle. The series that diverged most was left out of account in calculating the results. The animals were then given daily doses of 0.1 to 1.0 g/20 g of the thyroid powder to be tested, for 3 weeks, after which the carbon dioxide output was determined in the same way. If the animals had lost weight in the course of the test, the amount of carbon dioxide output was estimated to each gramme of the weight; if they had gained in weight, to each cm^2 of the surface area. The area was calculated from the formula: area $cm^2 = 11.36 \times (\text{weight in g})^{\frac{2}{3}}$. The percentual change in the consumption of carbon dioxide compared with the initial value shows the intensity of the effect of the thyroid preparation.

The test based on increased sensitivity to oxygen deficiency followed the method of SMITH, EMMENS and PARKES (1947): 2 different doses daily, 0.5 and 2 mg of each of the substances to be tested, were given during 1 week to mice weighing 18—20 g. Each dose was given to 20 mice. On the eighth day the test animals were put into an airtight glass vessel holding about 750 ml, and the time within which they were dead was registered. Twenty animals which had not been given any of the thyroid powder were used as controls. The determination of the efficacy of the preparations from the survival times of the animals followed the BURN (1950) method.

Reaction Based on Increased Sensitivity to Adrenaline. As described in the earlier study (PELTOLA 1950) the test animals were given thyroid powder in the form of water suspension of $2^1/_2$—5 per cent. The suspension was injected into a cube of bread given to the mice; they only got the rest of their food after eating the bread. Thyroid powder was given during 12 days, on the next day adrenaline was injected subcutaneously in the concentration 1 : 1,000 and the number of the dead animals was counted. 5 mg/20 g of each of the thyroid preparations was administered. Adrenaline was given in two ways: *I.* In increased doses to groups of 5 animals receiving the same thyroid preparation, namely 3, 4, 5, 6, 7, γ/g, and taking into account the amount of LD50 in estimating the efficacy. *II.* By giving 5 γ/g of adrenaline to groups of 20 animals of each thyroid preparation; the efficacy was then estimated from the mortality percentage of the animals which had been given adrenaline.

The clinical tests were carried out by giving the powder to be tested to 3 patients aged between 30 and 40 years, who had all been using thyroid preparations in treatment of hypothyroidism for 2 years at least, and for whom the dose had not been changed within this time and had proved adequate. In 2 patients the deficiency of the thyroid hormone was

due to a preceding thyroidectomy and in 1 to genuine hypothyreosis. All the patients had been taking a preparation called "Thyreoidin" (Orion OY), made of a commercial American powder. According to the manufacturers' analysis the amount of organic iodine in a 0.1 "Thyreoidin" tablet is 0.04 mg (0.02 g of the dry substance). The daily dose of "Thyreoidin" was 0.2 for one and 0.3 for two of the patients. Before the beginning of the test the basal metabolism of each of the patients was determined by Krogh's method, their weight, blood cholesterol and serum-bound iodine were registered. Then each of them was given a weighed amount of thyroid powder corresponding to the dose of the thyroid preparation they were in the habit of using, daily during 4 weeks, at the end of which period the same tests were again carried out. After this the patients went on taking their accustomed thyroid preparation for 4 weeks and the tests were repeated by giving to each, this time, some other thyroid preparation to be tested. The procedure was repeated twice. Thus the efficacy of each preparation was studied on all three subjects. The results were obtained by comparing the efficacy of the substance to be tested with the thyroid preparation habitually used by the patients, which thus was the same throughout.

Results.

Chemical Studies.

Table 1 gives the results obtained on the basis of the iodine determinations. The powders 1 and 2 thus contained practically the same amount, No. 3 perhaps slightly less iodine. As the error of the method is about 10 per cent, the difference is not statistically significant.

Table 1.

Iodine concentration in the dry substance of different thyroid preparations.

	Iodine Concentration in Dry Substance
Preparation No. 1 (Danish)	0.235 %
Preparation No. 2 (American).....	0.23 »
Preparation No. 3 (Finnish).....	0.22 »

Biological Studies.

Acetonitrile Test. — The results are shown in Table 2. Mortality varied considerably in the different test groups but without clearly conforming to any rule. There are dead animals in the group which was given 3/4 of the lethal dose, while on the other hand a 5/4 dose did not occasion a single death in the same group (*e. g.* prepa-

Table 2.

Mortality rate of the test animals in the acetonitrile test. The amount of thyroid powder suspension was equal for all the animals, 0.2 ml/daily on 3 days. Fraction: dead animals/total number of animals.

Dose of Acetonitrile, ml of 9 p. c. solution	Concentration of Thyroid Powder Suspension								
	No. 1			No. 2			No. 3		
	4,5 %	3 %	2 %	4.5 %	3 %	2 %	4.5 %	3 %	2 %
0.15 (= 3/4 LD)	1/2	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2
0.2 (= LD)	0/2	0/2	1/2	1/2	0/2	1/2	0/2	1/2	0/2
0.25 (= 5/4 LD)	0/2	1/2	0/2	0/2	0/2	1/2	1/2	0/2	1/2

rations Nos. 1 and 2). Likewise, dead animals occur among those who were given larger doses of thyroid though none are found among those who were given less, notwithstanding the fact that administration of thyroid powder should increase resistance to acetonitrile.

Test Based on the Determination of the Carbon Dioxide Output. The results appear in Table 3. We note that they vary considerably. No definite and consistent picture is obtained, but the results seem to imply that the preparations 1 and 2 are approximately of the same effect, No. 1 perhaps more effective and No. 3 somewhat less effective.

Table 3.

Increase of carbon dioxide output in time unit calculated to each g or cm² of surface area. See the text.

Dose of Thyroid Powder	Percentual Increase of CO_2-Output		
	No. 1	No. 2	No. 3
0.1 mg/20g	4 %	8 %	7 %
0.2 »	18 %	22 %	32 %
0.3 »	32 %	35 %	25 %
0.5 »	50 %	38 %	32 %
1.0 »	80 %	65 %	49 %

Test Based on Increased Sensitivity to Oxygen Deficiency. The results are given in Table 4. As far as any differences exist, preparations 1 and 2 again seem to be of equal efficacy and No. 3 somewhat stronger. Yet considerable variation occurs in the single test series, and the authors of the method themselves claim that the efficacy can be determined only within limits of 70—140 per cent differences.

Table 4.

Test based on increase of sensitivity to oxygen deficiency. Survival time of animals in airtight vessel holding 750 ml, after 8-day administration of thyroid powder. The longest and shortest survival time in each group is given in brackets.

Dose of Thyroid Powder	Average Survival Time of Animals			Control Animals
	No. 1	No. 2	No. 3	
—				123 min. (87—152 min.)
0.5 mg	116 min. (92—172 min.)	109 min. (74—154 min.)	104 min. (79—147 min.)	
2.0 »	96 min. (62—135 min.)	95 min. (41—173 min.)	90 min. (68—131 min.)	

Test Based on Increased Sensitivity to Adrenaline. Table 5 gives the results. When 5 mg/20 g of the preparation are given during 12 days, powder No. 1 causes a reduction to 4 γ/g in the LD50 of adrenaline, powder No. 2 to 5 γ/g and No. 3 to 6 γ/g. The mortality rate in a 20-animal series (Table 6) is lowest with powder

Table 5.

Test based on increase of sensitivity to adrenaline. Administration of thyroid preparations in doses of 5 mg/20 g on 12 days. The adrenaline dose injected subcutaneously. Fraction: Dead animals/total number of animals.

Thyroid Preparation	Adrenaline Dose							LD 50 γ/g
	3 γ/g	4 γ/g	5 γ/g	6 γ/g	7 γ/g	8 γ/g	9 γ/g	
—			1/5	2/5	3/5	4/5	4/5	7
No. 1	1/5	3/5	4/5	4/5	4/5			4
No. 2	0/5	1/5	3/5	4/5	5/5			5
No. 3	2/5	1/5	2/5	3/5	4/5			6

Table 6.

Test based on increased sensitivity to adrenaline. 5 mg/20 g of thyroid preparations was given on 12 consecutive days. 5 γ/g of adrenaline. Fraction: dead animals/total number of animals.

Thyroid Preparation	Deaths	Mortality Percentage
No. 1	19/25	76 %
No. 2	19/27	70 »
No. 3	12/24	50 »

No. 3, Nos. 1 and 2 occasion roughly the same mortality, No. 1 perhaps a little higher.

Clinical Studies.

The results are shown in Table 7. Preparation No. 1 causes a reduction in the weight and blood cholesterol concentration of all three patients, and an increase in the amount of protein-bound iodine, which would imply increase in the effect of the thyroid hormone. The basal metabolism is accelerated in two patients and remains unchanged in one. From the results it seems evident that preparation No. 1 is stronger in effect than the thyroid preparation habitually used by the patient. Preparation No. 2, on the other hand, is approximately equal in efficacy to the "Thyreoidin" preparation. When preparation No. 3 is used the weight of all three patients increases as does the blood cholesterol, while basal metabolism and the amount of the protein-bound iodine show reduction. The changes point to deficiency of the thyroid hormone, so that preparation No. 3 must be regarded as weaker in its effect than the "Thyreoidin" preparation. It may be noted, further, that the amount of the protein-bound iodine follows closely the variations registered in other studies, more closely than for instance those noted in basal metabolism.

Table 7.

Effect of the thyroid preparations on three patients with hypothyreosis. The comparison was drawn between thyroid powders and the "Thyreoidin" preparation (Orion OY). Each preparation was given for 1 month, after which, for 1—2 months, the patient's habitual dose of "Thyreoidin" was always administered.

Patient	Thyroid Preparation and Daily Dose	Weight kg	BMR %	Blood Cholesterol mg %	PBI γ%	Effect Compared with Thyreoidin
I	Thyreoidin 0.2 (= 0.04 g dry subst.)....	69	+ 5	212	6.4	
	No. 1 0.04 »	68	+ 12	190	8.8	No. 1 >
	» 2 0.04 »	68	+ 10	220	5.3	No. 2 =
	» 3 0.04 »	70	± 0	240	3.3	No. 3 <
II	Thyreoidin 0.3 (= 0.06 g dry subst.)....	76	+ 12	238	6.7	
	No. 1 0.06 »	74	+ 10	195	8.9	No. 1 >
	» 2 0.06 »	75	+ 15	230	6.6	No. 2 =
	» 3 0.06 »	78	— 5	285	2.6	No. 3 <
III	Thyreoidin 0.3 (= 0.06 g dry subst.)....	83	+ 4	223	5.2	
	No. 1 0.06 »	80	+ 18	180	8.0	No. 1 >
	» 2 0.06 »	82	+ 10	235	5.8	No. 2 =
	» 3 0.06 »	85	— 3	248	2.8	No. 3 <

Discussion.

The studies carried out showed very little difference in the iodine contents of the three different thyroid powders, in spite of their diverse origins.

In studying the biological efficacy of these preparations by different methods it should first of all be noted that the results obtained in the acetonitrile test are very unhomogeneous. The disparity of the results mainly seems to point to the great inaccuracy of the reaction when carried out in this manner. Considering that the lethal dose of acetonitrile may vary as much as 30 times in consequency of variation in diet alone, and is further dependent on such factors as the food intake of the test animals, light etc., it seems evident that the results obtained in the studies reflect the influence of various disturbing factors. The reaction in fact has been considered unsuitable as a standardizing method (GELLHORN 1923, GOLDNER 1928, KREITMAIR 1928, GADDUM 1931). The main point at which criticism has been directed is its non-specificity; in a species as closely related to the mouse as the rat reduction of resistance due to the effect of thyroid preparations is noted. The greatly variable range of errors is another disadvantage.

Great variation is revealed also in the reaction based on the carbon dioxide output. Yet the results point to a slightly weaker effect of powder No. 3 and perhaps to a stronger one of No. 1. No doubt the small number of the test animals (15) accounts for the difficulty in obtaining a reliable picture of the efficacy of the different substances. A larger series might perhaps improve the accuracy. JACOBSEN (1949), for instance, finds that only as large a series as 80—100 animals would be sufficient for one determination. In view of the number of animals and complexity of apparatuses required and the complicated nature of the execution the method must be considered very cumbersome.

For the test based on increase of sensitivity to oxygen deficiency, powder No. 3 is found to be somewhat more effective. Yet the differences are not significant. The relatively easy execution of the test must be taken as an advantage. The results, however, may show a very wide range of variation: in the same series, for instance, the survival time of one animal may be twice that of another, as appears from Table 4. Quite naturally the restlessness of the animal in the vessel may increase the consumption of oxygen

substantially (SMITH et al. 1947), which again reduces the oxygen content in the vessel. The death of the animals is not caused by an absolute reduction in the oxygen amount below a certain level, but by increase of sensitivity to deficiency of oxygen. So due to the effect of the thyroid powder the test animals die in an almost twice larger oxygen concentration than normal ones (SMITH et al. 1947), and a reduction of it may essentially impair the accuracy of the reaction because of restlessness of animals.

For the test based on increased sensitivity to adrenaline the results obtained by two methods are in approximate agreement. They show less efficacy in powder No. 3 and perhaps a larger efficacy in powder No. 1 compared with preparation No. 2. The technically easy and simple execution, which allows for the use of a large number of test animals and thus adds to its accuracy, is an obvious advantage. Moreover, in this reaction there were no noteworthy errors by the test animals through restlessness or diet.

Clinical studies point to a roughly similar result as the tests based on determination of carbon dioxide and sensitivity to adrenaline, which is evidence of the great specificity of these reactions. It should be noted that the protein-bound iodine apparently is an accurate exponent of the efficacy of thyroid preparations. Changes occurring in it correspond most clearly with those revealed in the biological tests, bringing out, for instance, in the tests based on carbon dioxide output and increased sensitivity to adrenaline a difference as to effect between preparations 1 and 2, which difference is not brought out, for instance in basal metabolism determinations. The latter actually is dependent on a great number of factors.

The determination of protein-bound iodine should therefore be taken into account as an aid to standardization, using it on patients whose thyroid hormone deficiency has remained on the same level over long periods of time.

The tests also show that in spite of the only slight divergences in the total iodine amount of different preparations, differences can be brought out in the biological effect of these drugs. The differences can be displayed both by certain tests on animals and on clinical material. Determination of the biological efficacy of thyroid preparations therefore remains an important measure, as was pointed out by Nordiska Specialitetskommissionen in 1932. These studies should not be restricted to determinations of the total iodine alone, as has been done in several pharmacopoeiae.

Summary.

The purpose of the studies was to test the accuracy of a new method of standardization based on the increased sensitivity to adrenaline of mice which is occasioned by thyroid powders. Three different thyroid preparations with practically identical total iodine content were used.

The biological efficacy of the preparations was compared by several biological methods of standardization. The acetonitrile method according to GRAB gave no consistent results. The MÖRCH method, based on the measurement of output of carbon dioxide, and the method to be tested, which rests on increased sensitivity to adrenaline, brought out differences in biological efficacy which are in agreement with results obtained in clinical studies. In the results obtained by the method based on increased sensitivity to oxygen deficiency the differences mentioned above are not visible.

In clinical studies, the amount of serum protein bound iodine is found to follow sensitively other variations that occur in the clinical picture when different preparations are used. The reaction must therefore be considered a good exponent of the efficacy of the thyroid preparation. It is a serviceable medium for checking the results of standardization obtained in animal experiments on human patients suffering from hypothyreosis.

The standardization method described in the present paper, developed on the basis of increase in sensitivity to adrenaline caused by thyroid powder, must be considered biologically specific, and the results obtained by it agree with clinical results. Technically the method is very simple and the possibility of errors is small.

References.

BARKER, S., HUMPHREY, M., and SOLEY, M., J. Clin. Invest. 1951. *30*. 55.

BOMSKOW, C., Methodik der Hormonforschung. I, Leipzig 1937.

BURN, J. H., Biological Standardization, Oxford 1950.

GADDUM, J., Quart. J. Pharmacol. 1931. *4*. 183.

GELLHORN, E., Pflüg. Arch. Ges. Physiol. 1923. *200*. 571.

GOLDNER, M., Z. klin. Med. 1928. *108*. 110.

GRAB, W., Arch. exp. Path. 1932. *167*. 313.

HARINGTON, C., and G. BARGER, Biochem. J. 1927. *21*. 169.

HUNT, R., J. Biol. Chem. 1905 *1*. 33.
— J. Pharmacol. 1910. *2*. 15.
— Amer. J. Physiol. 1923. *63*. 257.
— Arch. Int. Med. 1925. *35*. 671.
JACOBSEN, E., Personal communication 1949.
KENDALL, E., J. Biol. Chem. 1919. *39*. 125.
KREITMAIR, H., Z. ges. exp. Med. 1928. *61*. 202.
MÖRCH, K., Dansk Tidskr. Farm. 1928. 281.
Nordiska Specialitetskommissionens Årsberättelse 1932.
PELTOLA, P., Ann. Med. Exp. Biol. Fenn. 1950: *28*. Suppl. 4.
SMITH, A. U., C. W. EMMENS, and A. S. PARKES, Endocrinology. 1947. *5*. 31.

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Studies on Circulatory, Respiratory and Thermal Adaptation During Heavy Exercise

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It is a well known phenomenon that in the course of physical work and sports of endurance there occur distinct changes in the subjective pleasantness and in the objectively measurable efficiency. A good example among sports is skiing. At the beginning of the exercise a short — from a few seconds to a few minutes — adaptation phase is observed, during which the performance feels difficult. After this the organism attains a certain equilibrium, and the subjective feeling becomes rather pleasant. A first "steady state" (SS) has been attained (Hill 1922, Bock 1928, Knoll et al. 1937, Sachs 1937, Prokop 1948 and Bishop et al. 1953). After a time which depends on environmental conditions and on the training of the person in question and which varies within wide limits, a crisis "dead point" ("toter Punkt", DP) follows (Ewig 1926, Knoll et al., Meythaler 1937, Prokop), during which the work feels hard and unpleasant, and its intensity tends to fall. A final exhaustion is not yet, however, reached, since if the work will be continued with exertion of will the crisis will pass off in a few minutes. The following phase is called "second wind" or "second steady state" (SW) (Cook and Pembrey 1913, McKeith et al. 1921, Knoll et al., Meythaler, Prokop). Now the work goes on better again, and the worker is "warmed to the work". Not until later the real exhaustion follows which, according to the general opinion, is caused by the drying up of the glycogen depot. The terms meaning these different phases have been used

with some confusion, but the way they are used here may now be generally approved.

Many investigators have tried to analyse the reasons leading to the appearance of the "dead point" both through speculations and experiments. The experimental investigations have partly given conflicting results and have not as yet been able to elucidate the mechanism of DP. The significance of *respiratory gases* in the causation of DP has been studied by COOK and PEMBREY and MCKEITH et al. who observed an increased CO_2 excretion before SW and considered that CO_2 is the main factor in the attaining of DP. MIHAILA (1937) has observed several rises and falls in tidal volume and frequency and claimed that there is not one "dead point", but a crisis period with a succession of changes. According to him after the crisis the ventilation becomes deeper and the frequency slower. Further on he has shown that the respiratory quotient rises during the crisis which is due to the increased CO_2 excretion. According to PROKOP the CO_2 concentration of blood is at its lowest during DP. A reason for this phenomenon he considers a fall of the blood pH and hyperventilation which both have an increasing effect to CO_2 excretion. EWIG, among others, thinks that DP is caused by lack of oxygen in tissues and blood, but PROKOP has shown that the O_2 saturation of blood is at its highest in DP, because of the hyperventilation mentioned above. Instead, he thinks that the utilization of O_2 by the tissues would be somehow hampered.

The rôle of *blood sugar* in the causation of DP has been studied among others by JONESCU (1937), KNOLL and LÜSS (1934), and MEYTHALER (1937), all of whom think that hypoglycaemia is the reason for the phenomenen. PROKOP, however, has obtained contrary results observing that the blood sugar in DP is higher than normally. His results are supported by the findings of other investigators on exercise hyperglycaemia (*e. g.* CHRISTENSEN 1931). MEYTHALER seems to mean with DP the final exhaustion, which would explain his different results. — Many investigators have paid attention to the changes of *blood pH and lactate concentration* during exercise. DULIGE (1937) has observed that the blood pH distinctly falls during exercise and the maximal change is observed during DP. According to PROKOP, the lactic acid concentration of blood is at its highest in DP. MCKEITH has noticed during DP an increased excretion of lactic acid and chloride in urine and sweat and a distinct oliguria at the end of DP. He states that

sweating begins at the same time as the second wind. BERNER et al. (1926) claim, on the other hand, that sweating begins before the crisis. Further, they make the rather strange claim that the rectal temperature has not quite gone up when coming to the second wind, but that the axillar temperature has gone up. Already COOK and PEMBREY, however, observed that the "internal temperature" generally rises in work. DILL et al. (1931) claim that first in work the increase of body temperature is nearly even and then a constant temperature may be reached if the conditions allow it. NIELSEN (1938) has noticed that the body temperature in the same work always rises to the same higher level in spite of large environmental variations. According to BERNER the second wind appears the more quickly, the higher the external temperature and the harder the work.

About the reactions of the circulatory system in crisis only scarce information has been published. It is well known that the pulse and the systolic blood pressure rise during exercise, but the writer is not aware of any studies on their changes during DP. Tachycardia, however, is mentioned as a self-evident criterion of DP (KNOLL et al. etc.). The reactions of the peripheral circulation in DP have not been studied as far as is known to the writer. — LOWENTHAL et al. (1952) observed that when applying a relatively light work on the legs, the circulation of the hand increased distinctly, but that of the arm just a little or not at all. The load, however, was so small that no "dead point" was observed. CHRISTENSEN et al. (1942) claim that at the beginning of heavy work an instantaneous and considerable reduction of the skin's circulation always occurs for some minutes.

The theories about DP do not give any conclusive answer to this question. Hypotheses about excessively high carbon dioxide concentration and too low oxygen concentration in the organism during DP can be considered obsolete, and similarly it is quite unlikely that hypoglycaemia would cause the DP. On the other hand, it is apparent that the lactic acid concentration of blood has increased and the pH of the blood fallen during DP. Without doubt, this can be significant in the development of DP, but it is possible that there are other important factors too.

The purpose of the present study has been to try to elucidate the rôle of some factors in the attaining of DP. The changes of ventilation and oxygen consumption have been studied in the course of exercise, and on the basis of them the ventilation equiva-

lent for O_2 has been determined at different stages of exercise. In addition, attention has been paid to changes in rectal temperature. Further, the adaptation of the circulatory system has been investigated by measuring the pulse, systolic blood pressure and peripheral circulation at different stages of exercise.

Methods.

The subjects were three healthy male medical students. One of them had, at the time preceding the tests and during them, participated in some sports and done some manual work, the others almost nothing. Experiments were made during several months, on each subject ca. 25 times, but never more than 1 test a day. The results of 44 experiments are presented in this study. The tests were generally performed in the afternoon, and before them the subjects had been fasting for at least 3 hours. The tests were performed at ordinary room temperature (+ 20—22° C) except 3 tests which were done at + 12° C. Exercise was administered with the aid of a bicycle ergometer. In the preliminary experiments a load of ca. 1,100—1,300 kgm/min. was found suitable. At first the DP was attained in this experimental arrangement after ca. 20 minutes' work, but gradually the subjects became trained, and the average time in all the tests came to be 24 min. (range 17—35 min.). In the pulse and blood pressure experiments besides the ergometer a horizontally moving treadmill (ca. 11 km/hour) was used, by means of which DP was obtained more quickly.

Observing the crisis was both objectively and subjectively easy. Objectively it could be noticed that the speed of the ergometer tended to fall and the subject began to show incoordinated body movements. Breathing occurred more through the mouth, and the subject was dyspnoeic. The expression of the face was distressed and the face and the lips turned cyanotic. Sweating began distinctly before the crisis at ordinary temperature, as BERNER has also observed, although many previous investigators have claimed that sweating begins at the crisis (KNOLL et al., MCKEITH, PROKOP etc.). — Subjectively, the following phenomena were experienced by different subjects: A general feeling of weakness and exhaustion, which specially is felt in the acting muscle groups, and strong dislike for continuing the work. Then, the subject feels there is not air enough. The mouth feels dry. In the most strenuous treadmill tests there was a stitch on the side. — The crisis became quite distinct in ca. 1 minute and came to an end almost as distinctly when the performance was continued. The subjective condition became calm again and rather corresponded with the circumstances preceding the crisis. Most distinct was the subjective reappearance of taste for work. Fig. 1 presents a series of photographs showing the expressions of the face at different stages.

All the ventilation studies were performed with a Knipping spirograph (Type 157, Albert Dargatz, Hamburg), by means of which

Fig. 1. A series of photographs taken during a bicycle ergometer experiment. The first is taken in SS, the second in DP and the third in SW.

oxygen consumption, respiration volume and frequency were determined in different phases of exercise, and with the aid of these values the ventilation rate (l/min.) and ventilation equivalent for O_2 which means the relation of respirated air volume (in litres) to the corresponding oxygen consumption (in hundreds of ml) were calculated. It is not possible to determine the CO_2 production with a Knipping spirograph, and this was not considered necessary in view of the previous clearcut results. — The internal body temperature was measured by an electric thermometer per rectum. — The pulse values were obtained by performing double counts, with palpation on the neck and auscultation on the heart. — The systolic blood pressure was measured from the right arm with a Hg-manometer and manchette. The systolic pressure only was taken, as the accurate measurement of the diastolic pressure during exercise is not possible with auscultation. All the measurements mentioned above could be done during work. — The peripheral circulation was studied with plethysmographs at the same time from the right hand and the left forearm. In these tests a plethysmograph described by BARCROFT and SWAN (1952) was used. Their instructions were exactly observed in the experimental procedure. The hand and forearm of the subject were kept closed in the apparatus during the whole exercise and firmly supported. During registering the subject was, however, compelled to interrupt pedalling the ergometer for 20—30 seconds.

The first observation was generally, except for ventilation experiments, made at the beginning of the experiment before the work began, then 2 observations were made in the steady state — at 5 and 15 minutes — the following in DP, then one at the beginning of the second wind, and the last 5 minutes later, after which the test was finished.

Results.

In the ergometer tests the first "steady state" came to an end after appr. 24 minutes from beginning the experiment, as mentioned above. DP lasted appr. 4 minutes, varying from 2.5

The means and their standard errors of the results obtained in 10 ventilation experiments.[1]

The means and their standard errors of the results obtained in 10 ventilation experiments.[1]

	"1st steady state"		DP	"second wind"	
Oxygen consumption (l/min)	1.66 ± 0.09	1.78 ± 0.11	2.00 ± 0.12	1.83 ± 0.12	1.83 ± 0.17
Tidal volume (l)	1.95 ± 0.18	2.12 ± 0.18	2.34 ± 0.13	1.98 ± 0.18	2.04 ± 0.28
Frequency of breathing (/min)	23.0 ± 1.4	26.5 ± 1.5	33.5 ± 0.83	29.1 ± 1.6	29.3 ± 1.2
Ventilation minute volume (l/min)	43.7 ± 3.7	54.3 ± 3.0	78.1 ± 3.9	55.7 ± 3.0	58.9 ± 4.9
Ventilation equivalent for O_2 (l/100 ml)	2.62 ± 0.13	3.09 ± 0.12	4.01 ± 0.26	3.08 ± 0.13	3.32 ± 0.23

[1] Because of lack of space, the original observations are not documented in this paper; copies of them are available on application from the writer.

Table 2.

The differences, their standard errors and statistical significance between successive observations calculated as a percentage from the value at the "dead point" in ventilation experiments.

	"1st steady state"		DP	"second wind"	
	1	2	3	4	5
	2—1	3–2		4—3	5—4
Oxygen consumption	+ 5.9 ± 4.9 t = 1.2, p > 0.05	+ 10.0 ± 3.8 t = 2.6, p < 0.02		— 8.0 ± 2.6 t = 3.1, p < 0.01	— 2.9 ± 3.5 t = 0.8, p > 0.05
Tidal volume	+ 7.0 ± 6.7 t = 1.0, p > 0.05	+ 10.1 ± 3.7 t = 2.7, p < 0.02		— 16.5 ± 3.5 t = 4.7, p < 0.01	+ 1.9 ± 4.8 t = 0.4, p > 0.05
Frequency of breathing	+ 10.6 ± 5.8 t = 1.8, p > 0.05	+ 20.5 ± 4.2 t = 4.9, p < 0.01		— 12.8 ± 5.0 t = 2.6, p < 0.02	— 0.9 ± 6.5 t = 0.14, p > 0.05
Ventilation minute volume	+ 14.6 ± 3.7 t = 4.0, p < 0.01	+ 29.9 ± 2.3 t = 12.8, p < 0.01		— 28.2 ± 3.1 t = 9.2, p < 0.01	+ 2.2 ± 4.5 t = 0.5, p > 0.05
Ventilation equivalent for O_2	+ 11.4 ± 5.4 t = 2.1, p < 0.05	+ 21.5 ± 3.3 t = 6.5, p < 0.01		— 21.2 ± 4.1 t = 5.2, p < 0.01	+ 4.2 ± 5.8 t = 0.7, p > 0.05

to 6 minutes. The second wind lasted clearly up to the end of the test, and no exhaustion crisis was observed any more. — In the running tests DP was observed earlier, but otherwise the pulse and blood pressure observations gave results corresponding to those in the ergometer tests, and they are not reported separately.

Ventilation during exercise.

The results from 10 experiments are presented here. *Oxygen consumption* in steady state after 5 min. from beginning the exercise was on an average 1.66 ± 0.09 (standard error of the mean) l/min. and at 15 min. 1.78 ± 0.11 l/min. In DP the absorption of oxygen had increased to 2.00 ± 0.12 l/min. and after its disappearance, during the second minute of SW, it fell to 1.83 ± 0.12 l/min. remaining the same until the end of exercise (Table 1). As the absolute values in separate subjects were at somewhat different levels depending on individual variations, a clearer picture of the relative changes during exercise is obtained from the percentage values expressed. Therefore, all the readings were changed into percentages, taking the reading in DP as 100. The significance of the differences between separate means was calculated on the basis of these values. The rise in oxygen consumption between the first and the second observation was not significant. But the rise was significant when coming to DP and likewise the fall at the beginning of SW, after which no significant change was observed (Table 2).

The tidal volume was correspondingly 1.95 ± 0.18 l and 2.12 ± 0.18 l during the steady state. In DP it ascended to 2.34 ± 0.13 l, falling down clearly at the beginning of SW. At the end of the exercise the situation was rather the same (Table 1). Also these results were changed into percentage figures as before in order to eliminate the individual differences, and the significance of differences was determined from them. During SS the change of the tidal air was not significant. In DP the tidal volume increased so much that the difference was significant. Still clearer was the reduction of respiration volume at the beginning of SW. No significant change occurred after this (Table 2).

The frequency of breathing showed a similar sequence of changes like both previous factors. In SS the frequency was first 23.0 ± 1.4 times a minute and then 26.5 ± 1.5/min. A distinct rise occurred in DP, when the frequency was 33.5 ± 0.8/min. and a smaller

Table 3.

Table 3.

The means and their standard errors of 10 rectal temperature, pulse rate and systolic blood pressure observations and of 12 forearm blood flow and hand blood flow observations.

	Rest	"1st steady state"		DP	"Second wind"	
Rectal temperature (C)	37.40 ± 0.17	37.93 ± 0.16	38.27 ± 0.15	38.57 ± 0.15	38.59 ± 0.13	38.66 ± 0.32
Pulse rate (/min.).....	71.6 ± 2.7	134.5 ± 2.8	148.4 ± 4.6	159.8 ± 5.0	156.0 ± 4.5	155.7 ± 4.9
Systolic blood pressure (mm Hg)...	120.5 ± 3.0	159.4 ± 4.1	168.0 ± 3.6	171.0 ± 5.1	163.0 ± 4.2	158.5 ± 4.1
Forearm blood flow (ml)	0.97 ± 0.18	1.85 ± 0.16	2.63 ± 0.15	3.16 ± 0.19	3.14 ± 0.20	3.24 ± 0.25
Hand blood flow (ml)	5.45 ± 0.39	8.01 ± 0.62	10.0 ± 0.70	12.2 ± 0.80	11.2 ± 0.71	10.7 ± 0.80

Table 4.

The differences, their standard errors and statistical significance between successive observations calculated as a percentage from the value at the "dead point" in rectal temperature, pulse, blood pressure and peripheral circulation experiments.

	"1st steady state"		DP	"second wind"	
	0 1—0 1	2—1 2	3—2 3	4—3 4	5—4 5
Rectal temperature	+ 1.39 ± 0.54 t = 2.6, p < 0.02	+ 0.87 ± 0.38 t = 2.3, p < 0.05	+ 0.78 ± 0.17 t = 4.5, p < 0.01	+ 0.04 ± 0.09 t = 0.46, p > 0.05	+ 0.19 ± 0.13 t = 1.4, p > 0.05
Pulse rate	+ 39.5 ± 2.4 t = 16.3, p < 0.01	+ 8.4 ± 1.7 t = 4.8, p < 0.01	+ 7.1 ± 0.60 t = 11.7, p < 0.01	— 2.3 ± 0.75 t = 3.1, p < 0.01	— 0.3 ± 1.1 t = 0.26, p > 0.05
Systolic blood pressure	+ 21.7 ± 2.7 t = 8.1, p < 0.01	+ 5.1 ± 2.2 t = 2.3, p < 0.05	+ 1.5 ± 1.3 t = 1.1, p > 0.05	— 4.5 ± 1.6 t = 2.8, p < 0.02	— 2.6 ± 1.9 t = 1.3, p > 0.05
Forearm blood flow	+ 28.7 ± 5.2 t = 5.5, p < 0.01	+ 24.1 ± 5.7 t = 4.2, p < 0.01	+ 16.2 ± 2.7 t = 6.0, p < 0.01	— 0.6 ± 1.7 t = 0.35, p > 0.05	— 0.4 ± 3.4 t = 0.12, p > 0.05
Hand blood flow.....	+ 21.8 ± 4.3 t = 5.1, p < 0.01	+ 15.2 ± 5.0 t = 3.1, p < 0.01	+ 17.3 ± 3.7 t = 4.7, p < 0.01	— 7.4 ± 3.0 t = 2.5, p < 0.05	— 4.9 ± 3.9 t = 1.3, p > 0.05

fall at the beginning of SW (Table 1). During SS, the frequency increased between the first and the second reading to a statistically significant extent. A much more significant increase occurred when coming to DP. At the beginning of SW the frequency had fallen a little, but the change was not as highly significant as the former. Five minutes later there was only an insignificant fall (Table 2).

Ventilation minute volume is obtained by multiplying the tidal volume by the frequency, and thus the similar changes are still more distinctly observable. At the beginning of SS (5 min.) the ventilation was 43.7 ± 3.7 l/min., 10 min. later it had risen to 54.3 ± 3.0 l/min., and in DP it even rose to 78.1 ± 3.9 l/min. In SW it returned to the same level as before and did not vary much from that (Table 1). The differences between the phases of exercise calculated as percentage values came to be as follows: In SS a significant increase of respiration occurred, but it increased still more significantly when passing from SS to DP. An equal fall occurred between DP and SW. During SW there was no significant change any more (Table 2).

The ventilation equivalent for oxygen means the relation of the respirated air volume (in litres/min.) to the absorption of oxygen (in 100 ml/min.). It can be counted on the basis of the test results mentioned before. In SS it is first 2.62 ± 0.13 and then 3.09 ± 0.12. During DP it rose to 4.01 ± 0.26 and during SW it fell to 3.08 ± 0.13 and in the end to 3.32 ± 0.23. This shows that during DP the absorption of oxygen did not increase to as large an extent as the respiration, and consequently there existed an obvious hyperventilation. The increase of the equivalent in SS is significant. The increase from SS to DP is clearly significant. A significant fall occurred also when coming to SW, and no more change occurred in the end (Tables 1 and 2).

Rectal temperature.

The rectal temperature was at first measured at rest, the average being $37.40 \pm 0.17°$ C. Five min. after the beginning of the exercise the corresponding value was $37.93 \pm 0.16°$ C. The difference of these values as percentages is significant. During SS temperature rose to 38.27 ± 0.15, and the difference from the former is again significant. In DP temperature rose to $38.57 \pm 0.15°$ C, and the difference is more clearly significant than the former one. In DP the growth of temperature stopped. The values in SW

do not differ significantly from the DP value, Tables 3 and 4. — At room temperature sweating began during SS 10—15 minutes after the beginning of exercise, *i. e.* distinctly before DP, but it seemed to increase in the region of DP. Three exercise tests were performed at + 12° C, and in those DP came much later than in the others. In those, sweating clearly did not begin until the end of SS and during DP.

Pulse and blood pressure.

The average rest *pulse* was 71.6 ± 2.7, in SS it had, 5 minutes after the start, risen to 134.5 ± 2.8. This difference is, calculated on the basis of corresponding percentage values, highly significant. At 15 minutes the pulse was 148.4 ± 4.6, and the difference from the previous value is significant. A distinct rise to 159.8 ± 5.0 occurred also in DP, as compared to the second value of SS. The pulse fell significantly at the beginning of SW. Then the situation remained unaltered until the end of the test. These results were obtained in the ergometer tests (Tables 3 and 4), but corresponding variations were obtained on the treadmill.

About the variations of *blood pressure* at DP there exist no previous studies as far as is known. The average resting systolic pressure was 120.5 ± 3.0 mm Hg and it rose at the beginning of exercise to 159.4 ± 4.1 mm Hg.[1] The difference between the next value, 168.0 ± 3.6 mm Hg, and the former one is also statistically significant. Passing from SS to DP the pressure rose only to 171.0 ± 5.1, and the rise is not significant. At the beginning of SW the pressure fell to 163.0 ± 4.2 mm Hg which differs significantly from the DP value. The final value does not differ significantly from the former one. Blood pressure in SW fell, thus, to the same level where it was at the first half of SS, but it did not change between SS and DP (Tables 3 and 4).

Peripheral circulation.

During registering the upper limbs were to be kept quite motionless and relaxed which was not easy with the subject sitting on the saddle of the ergometer. The plethysmographic study of the forearm is a measure of circulation in resting muscles, the change in the hand volume, on the other hand, that of blood flow in skin (GRANT and PEARSON 1938). *The forearm blood flow*

[1] This difference is highly significant.

was at rest on an average 0.97 ± 0.18 ml/100 ml forearm volume/min. and 5 minutes after the beginning of exercise it had risen to 1.85 ± 0.16 ml. This difference, calculated again in percentages from the DP value, is statistically significant. In the end of SS the blood flow rose to 2.63 ± 0.15 ml and this value differs from the former significantly. In DP the blood flow rose to 3.16 ± 0.19 ml. Also here the difference from the previous value is significant. In SW both the observations gave rather the same result as in DP. Neither of them differs from DP or from each other significantly (Tables 3 and 4).

The hand blood flow showed typical changes going in the same direction in majority of experiments, but by one subject both typical and in two tests also "atypical" results were observed in DP. Except these 2 observation series the following results were obtained: At rest the hand circulation was appr. 5.45 ± 0.39 ml/100 ml hand volume/min. and it rose at 5 minutes to 8.01 ± 0.62 ml and the percentage rise was clearly significant. After 15 min. of exercise the blood flow had increased to 10.0 ± 0.70 ml, differing again from the former value significantly. During DP the circulation further increased to 12.2 ± 0.80 ml and this rise is also significant. During SW the hand circulation fell to 11.2 ± 0.71 ml and the difference is significant but less obviously than earlier. The value obtained 5 minutes later does not differ from the former one significantly. In the two clearly exceptional tests a transient fall occurred in hand circulation during DP. There are several possible explanations to this exceptional behaviour. Either the subject in question could not keep his hand quite relaxed and motionless during the recording or an individually different reaction during the crisis occurred (Tables 3 and 4).

Discussion.

On the basis of the results the phenomena examined here can be divided into three groups by using their behaviour in the "dead point" as a criterion. The first group is formed by the changes which are typical in DP as the observed reedings obviously differ from the corresponding ones in the "1st steady state" and in the "second wind". All the measures obtained in the ventilation experiments — O_2 consumption, tidal volume, ventilation frequency

as well as ventilation volume and ventilation equivalent for O_2 — increase in DP clearly above the level prevailing in SS and decrease again significantly in SW. Likewise the pulse rate reaches its maximum in DP and gets somewhat slower in SW. The blood flow of hand rises continuously from the beginning of the performance up to DP and diminishes in SW. The second group, according to the conduct in DP, is formed by the body temperature and the blood flow of forearm. They show a continuous tendency to rise up to DP, but in SW no change worth mentioning occurs. Thirdly the extraordinary behaviour of the systolic blood pressure is still noticed. It rises at the beginning of the work steeply and later in SS slowly. In DP no significant change occurs with regard to SS, but coming to SW the pressure regularly decreases.

An essential point seems to be that a change in the regulation of body temperature is connected with the appearance of DP. Then the temperature takes its place on the level assumed by the work quantity. The work remains the same as also its mechanical efficiency, because the consumption of O_2 is about the same in SW as in SS. The loss of heat has to increase through increased skin blood flow and perspiration in DP. The distribution of blood in the organism changes, and this may occasionally lead to slight symptoms resembling collapse. This hypothesis about the nature of DP is in accordance with the observation that the systolic blood pressure decreases after DP. This may be supposed to be caused by the opening of blood vessels in skin which then diminishes peripheral resistance of circulation. This hypothesis also harmonizes with the observation that DP begins later in cold than in warm environment (BERNER et al., own observations). It is quite likely that the final temperature level is reached later in cold temperature although this point has not been examined systematically here.

The collapse hypothesis is also supported by the present observations on peripheral circulation. The forearm blood flow rises up to DP and keeps nearly constant in SW. The forearm may be considered as representing the resting body musculature. This rise of circulation may, in the main, arise from an increase in body temperature, but a static tension of seemingly resting muscles may also increase their need of blood. The skin blood flow measured in the hand seems to rise continuously until DP and then, in SW, showed a slight tendency to fall. One subject

made an exception in two experiments in which a passing decrease occurred in DP. The rôle of the peripheral parts of extremities — of the first order effectors of heat loss — is not dominating any more after the perspiration has begun effectively on the surface of all the body. From this point of view a slight decrease of skin blood flow in the hand in SW is not at all surprising. If DP is regarded as an expression of a passing collapse, one has also to take into consideration that changes in skin blood flow are known to occur in both directions in collapse conditions (cf. changes in skin colour in connection with fainting).

A certain kind of collapse is indicated also by the ventilation and pulse rate. The observations show the appearance of hyperventilation in DP. According to GRAY (1950) the cause of work hyperventilation is not yet fully known. Perhaps receptors in muscles, tendons and joints, different chemoreceptors or central and peripheral thermoreceptors reflexively cause this rise of ventilation above the real need. GRAY verifies also that the ventilation equivalent for O_2 rises in heavy work. But he does not mention anything about the "dead point" phenomenon. An extra hyperventilation in DP in addition to the usual ventilation at work was observed in the present study. That may have been caused by acidosis in the blood (MCKEITH et al., DULIGE, ASMUSSEN and NIELSEN 1946, PROKOP), but also by anaerobic intermediates in muscles, from which they could act on the respiratory centre by reflex action. These observations show also that the ventilation equivalent reaches its maximum in DP; it means that the ventilation rises relatively more than the O_2 consumption. Thus the organism is, at least from the point of oxygen demand, ventilating over the actual needs. The same kind of mechanism could also cause that passing increase of pulse rate in DP. Possibly some hormonal factors also take part in the observed changes.

The abrupt passing of DP with disappearance of subjective tiredness and weakness in the working muscles and of other symptoms might be explained so that the temperature of muscles has reached the level corresponding best with the rate of chemical reactions in the muscles. In SS these may have to work in partly anaerobic conditions and thus acid products of intermediate metabolism could accumulate in muscles. At a sufficiently high temperature aerobical energetic reactions might again become dominating and the accumulation of acid products might cease.

This is supported by *e. g.* the claim that the lactic acid production of blood might, somewhat, decrease after DP (PROKOP).

In spite of all these and earlier examinations the problem of DP cannot yet be regarded as absolutely settled from every point of view. It seems quite evident that at the time of DP a new adaptation occurs in the circulatory system and also in the metabolism, and obvious changes take place in respiration. Whether there is an absolute coincidence of all these changes in different working conditions, remains to be shown.

Summary.

1) The behaviour of ventilation, rectal temperature, pulse rate, systolic blood pressure and peripheral circulation in man was examined during heavy exercise, taking special notice of the changes in the region of "dead point" ("toter Punkt", DP).

2) It was observed that the ventilation volume — both the tidal volume and the frequency — and the oxygen consumption reached their maximum values in DP, but the rise in ventilation was relatively greater than in oxygen consumption, and thus the ventilation equivalent for O_2 rose in DP (hyperventilation). The pulse rate and also the skin blood flow as measured in the hand reached their highest values in DP in the same manner.

3) The rectal temperature rose nearly equally until DP, but not any more in the "second wind". The blood flow in resting muscles as measured in the forearm changed in the same way.

4) The systolic blood pressure rose clearly at the beginning of exercise, but changed only a little later in the first "steady state" and DP. Then, in the "second wind" an obvious decrease occurred.

5) A hypothesis is presented according to which the essential point in the origin of DP is a slight collapse due to the adaptation of the circulatory system to new conditions of heat loss, when the body temperature during work stabilizes at its final level.

References.

ASMUSSEN, E. and M. NIELSEN, Acta Physiol. Scand. 1946. *12*. 171.

BARCROFT, H. and H. J. C. SWAN, Sympathetic Control of Human Blood Vessels, London 1953.

BERNER, G. E., C. C. GARRETT, D. C. JONES and R. J. NOER, Amer. J. Physiol. 1926. *76*. 586.

Bishop, J. M., K. W. Donald and O. L. Wade, J. Physiol. 1953. *122*. 30.
Bock, A. V., C. Vancaulaert, D. B. Dill, A. Fölling and L. M. Hurxthal, Ibidem 1928. *66*. 162.
Christensen, E. H., Arbeitsphysiol. 1931. *4*. 128.
Christensen, E. H., M. Nielsen and B. Hannisdahl, Acta Physiol. Scand. 1942. *4*. 162.
Cook, F. and M. S. Pembrey, J. Physiol. 1913. *45*. 429.
Dill, D. B., H. T. Edwards, P. S. Bauer and E. J. Levenson, Arbeitsphysiol. 1931. *4*. 508.
Dulige, E., Ibidem 1937. *9*. 430.
Ewig, W., Münch. Med. Wschr. 1926. *1925*. 1955.
Grant, R. T. and R. S. B. Pearson, Clin. Sci. 1938. *3*. 119.
Gray, J. S., Pulmonary Ventilation and its Physiological Regulation, London, Illinois 1950.
Hill, A. V., J. Physiol. 1922. *56*. 19.
Jonescu, A., Arbeitsphysiol. 1937. *9*. 424.
Knoll, W., Ibidem 1937. *9*. 414.
— and J. Lüss, Ibidem 1934. *7*. 517.
Lowenthal, M., K. Harpudar and S. D. Blatt, J. Appl. Physiol. 1952. *4*. 689.
McKeith, N. W., M. S. Pembrey, W. R. Spurrell, E. C. Warner and H. J. W. J. Westlake, J. Physiol. 1921. *55*. VI.
Meythaler, F., Klin. Wschr. 1937. *27*. 951.
— and A. Droste, Ibidem 1934. *24*. 439.
Mihaila, J., Arbeitsphysiol. 1937. *9*. 415.
Nielsen, M., Scand. Arch. Physiol. 1938. *79*. 193.
Prokop, L., Leibesübungen und Leibeserziehung 1948. *3*.
Sachs, J. and W., Amer. J. Physiol. 1937. *118*. 697.

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Glucuronic Acid Metabolism in Guinea Pigs.

By

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In most of the previous *in vivo* experiments carried out for elucidation of the metabolism of glucuronic acid dogs or rabbits have been used as test animals. In the studies recently made in this laboratory on glucuronic acid, the use of the guinea pig has seemed to offer some interesting aspects.

Contrary to the claims of O. RYGH and A. RYGH (1932), the ability of the guinea pig to synthesize glucuronic acid became partially evident already from the investigation by WIDMARK and GLIMSTEDT (1933), in which it was demonstrated that the urine samples of normal as well as scorbutic guinea pigs gave in most cases a positive naphthoresorcinol reaction, though in both groups the reaction might occasionally be negative for some reason. The observation that liver slices of at least the normal guinea pig are able to build glucuronic acid conjugates *in vitro* was already made in the experiments by LIPSCHITZ and BUEDING (1939). In other respects, however, it is difficult to find in the literature exact information on glucuronic acid metabolism in the guinea pig.

Theoretically the subject is of interest for the reason that the guinea pig is one of the few animals which are unable to synthesize l-ascorbic acid and which therefore can be successfully used as test animals in the study of scurvy. On the other hand it has been considered probable that glucuronic acid and l-ascorbic acid may have some relationship in respect to biological synthesis. In fact, ISHERWOOD, CHEN and MAPSON (1953) have reported the interesting observation that d-glucuronic acid and γ-lactone

of d-glucuronic acid, when fed into rats, was transformed into l-ascorbic acid.

With a view to the possible rôle of l-ascorbic acid as a factor in enzymatic reactions, the use of guinea pigs as experimental animals provides an opportunity to study to what extent l-ascorbic acid is an indispensable additional factor for linking the OH group to, for instance, camphor or other ring compounds, which are then excreted as a glucuronic acid conjugate in the urine.

The study of the glucuronic acid metabolism of guinea pigs is of further interest since scurvy seems to be associated with some disturbances in the relationship between connective tissue and polysaccharide-like substances.

One of the difficulties in the study of glucuronic acid metabolism has been the failure so far to develop a completely accurate and specific analytical micromethod. The colorimetric technique has usually been based on the naphthoresorcinol method of TOLLENS (1909). In the light of experiences obtained in the use of the naphthoresorcinol test in this laboratory it must be said that it undoubtedly is of relatively great value in comparison with other colour reactions in the colorimetric micromethod, but that its use for quantitative purposes is associated with numerous difficulties and error factors. Of the micromethods described in the literature, that of JARRIGE (1947) has appeared in the tests made in this laboratory quite serviceable because of its speed and simplicity. However, it is not suitable as such for the determination of the glucuronic acid content of the blood. On the other hand, a method that has been under development in this laboratory for blood determinations has proved serviceable for guinea pig blood and serum but in the case of rat blood it has so far given poorly reproducible results for some inexplicable reason.

Using the modified naphthoresorcinol method the values shown in Table 1 were obtained for the blood, serum and urine of guinea pigs on ordinary cage diet.

As is seen from this table, the glucuronic acid levels in whole blood and serum were of the same order of magnitude and did not greatly differ from the values reported in the literature for the glucuronic acid content of blood. The amount of glucuronic acid eliminated into the urine was, on the whole, of the same magnitude in relation to the unit of weight as that of the rabbit,

Table 1.

Occurrence of glucuronic acid in normal guinea pig.

Blood	3.5— 4.4 mg %
Serum	3.2— 4.9 » »
Urine	27 —95 » »[1]

[1] Excretion in 24 hr per 100 g body weight equal 1.1—2.2 mg.

considerably greater than that of man, but lower than that of the rat.

The form of occurrence of the glucuronic acid which can be determined in the protein-free blood and serum filtrates and in urine is a different matter. A part of it is usually present in the form of ordinary simple conjugates produced in the processes of detoxication, but a part of the demonstrated glucuronic acid may possibly be derived from some complexes of the carbohydrate type.

In order to shed some light on the extent to which the guinea pig is capable of producing, by endogenic glucuronic acid synthesis, glucuronic acid to form conjugates with some foreign substance, Table 2 shows the results of a few experiments with guinea pigs in which a study was made of the increase of the glucuronic acid level in the urine following subcutaneous application of camphor.

Table 2.

Effect of subcutaneous application of camphor.

The dosage of camphor in mg	Increase of glucuronic acid excretion in urine
100 mg	400 %
50 »	317 »
25 »	225 »

These experiments demonstrated that the guinea pig is capable of relatively powerful glucuronic acid conjugation following the administration of a foreign substance. At the same time it became evident that this animal, as also for instance the dog, is apparently able to convert camphor by hydroxylation to campherol, which then is glucosidally combined with glucuronic acid.

Among other preliminary experiments on the glucuronic acid metabolism of the guinea pig, mention may here by made of certain observations on the relationship between scurvy and the occurrence of glucuronic acid. These observations were made in the course of certain other systematic studies on scurvy in which the test animals were guinea pigs which had been kept on a vitamin C deficient diet. Some of the animals were completely without this vitamin and some were given ascorbic acid solution perorally to prevent the development of scurvy. The glucuronic acid concentrations in the blood and urine are shown in Table 3.

Table 3.

Effect of scurvy on serum levels (mg per cent) and excretion of glucuronic acid in urine (mg/100 g body weight in 24 hr).

	Controls		Scurvy	
	Mean	Range	Mean	Range
Serum	4.6	3.9—5.2	6.5	4.9—8.3
Urine	1.6	1.2—2.1	1.8	1.6—2.0

It is seen from these experiments that a deficiency of ascorbic acid caused at least no reduction in the glucuronic acid values. On the contrary, these preliminary experiments seem to point to some rising tendency in the serum glucuronic acid.

Reference may also be made in this connection to determinations of the serum mucopolysaccharide concentration carried out by two different techniques in connection with the scorbutogenic experiments. A certain tendency to increased mucopolysaccharide levels was seen in some of the cases of scurvy. It is not as yet possible to state any opinion on the connection between this event and the tendency to increased glucuronic acid levels observed in scurvy by the naphthoresorcinol method. These phenomena may probably be due to the presence in the organism of the scorbutic guinea pigs of some carbohydrate-like compounds which for some reason have not become joined to connective tissue.

It is the intention to study also the effect of scurvy on the production of glucuronic acid conjugates in the organism when guinea pigs are given ring compounds either containing the hydroxyl group or not containing this group, such as for instance

terpenes and transformation products of tryptophane. Because of technical difficulties this work has not been able to proceed beyond a preparatory stage.

An effort has also been made to observe how well the guinea pig, as compared to the rat, is capable of enzymatically transforming glucuronic acid and glucuronic acid lactone into other compounds.

Summary.

This report points out the more extensive biochemical significance to the elucidation of the production and mechanism of action of l-ascorbic acid which may be derived from a detailed study of the glucuronic acid metabolism of the guinea pig.

To supplement the deficient information in the literature on the glucuronic acid metabolism of the guinea pig, determinations were made of the glucuronic acid concentration of guinea pig whole blood and serum and the total amount of glucuronic acid daily eliminated into the urine. It was demonstrated that when necessary for the detoxication of a foreign substance the guinea pig is capable of very considerably increasing the glucuronic acid synthesis. In studying the relationship between scurvy and glucuronic acid metabolism it was observed that scurvy seems to have at least no reducing effect on the normal concentrations in the blood and urine. On the contrary, it appeared probable that a tendency to somewhat increasing serum glucuronic acid levels occurred in scurvy.

References.

Artz, N. E. and E. M. Osman, Biochemistry of Glucuronic Acid. New York 1950.

Isherwood, F. H., Y. T. Chen and L. W. Mapson, Nature 1953. *171*. 348.

Jarrige, P., Bull. soc. chim. biol. 1947. *29*. 461.

Lipschitz, W. L. and E. Bueding, J. Biol. Chem. 1939. *129*. 333.

Rygh, O. and A. Rygh, Z. physiol. Chem. 1932. *211*. 275.

Tollens, C., Ibidem 1909. *61*. 95.

Widmark, E. M. P. and G. Glimstedt, Ibidem 1933. *215*. 147.

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Rhythmical Potential and Impedance Variations in Isolated Frog Skin induced by Lithium Ions.

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A vast literature exists on the electrical behaviour of frog skin. In the early periods this was chiefly concerned with the effects of different ions in different concentrations on the potential across the skin. In recent years, however, the interest has turned to the nature of the ionic transport processes, which are underlying the electrical "symptoms" of the vital functions of the skin. It is now realized, that the electrical potential and conductance are related to an "active transport" of, in the main, the sodium ions from outside to inside of the skin. We owe this knowledge to a series of excellent research works, of which those made by HUF (1935—1953), KROGH (1937, 1946), USSING (1949—1953) and LINDERHOLM (1952) are especially worth mentioning (for a full review of relevant papers see LINDERHOLM 1952, p. 35—38).

Already in 1904 GALEOTTI had found that only sodium and lithium salts could maintain a high potential (the outside reckoned as negative), while other salts like those of potassium caused low or zero potentials. Similar results, although extended over a wide range of concentrations, were observed among others by HASHIDA (1922), MOTOKAWA (1935, 1938) and GREVEN (1941). MEYER and BERNFELD (1946) and TEORELL (1946, 1949) could confirm the "depolarizing" effect on the potential of KCl placed

outside of the skin. The last author also demonstrated a concomitant rise of the A. C. impedance (l. c. Table I).

The early findings cited above, namely that only Li ions could take the place of Na ions with respect to the potential effects, in conjunction with similar observations on the excitability of muscles (OVERTON 1902), axons and nerves (HODGKIN and KATZ 1949, HUXLEY and STÄMPFLI 1951, LUNDBERG 1951—52), suggest the possibility of using Li as an "isotope" of Na in studies on the problems of electrogenesis of living tissues. In the course of such experiments on the isolated frog skin, performed by aid of a refined, continuous recording technique to be described below, the interesting observation has been made that the *skin potential* varied *rhythmically* over long periods when the skin was bathed *with 20—300 mN LiCl* on the outside. The rhythm was more or less sinusoidal with an amplitude of the order 0.1 to 10 millivolts and a regular frequency of about 3 to 10 oscillations in a 10 minute period. Approximately parallel variations in the *low frequency A. C. conductance* were also observed.

The induction of "quasi-periodic" oscillations of the skin *potential* by Li salts was reported already by HASHIDA and studied in considerable details by his pupil TAKENAKA (1937). They found, among other things, that the Li ions were effective only when administered on the anatomical outside of the skin, the oscillations were rather insensitive to pH, but could be abolished by certain anaesthetics. As the experiments of the Japanese workers were not designed to meet the requirements demanded by the modern concepts on the electrogenesis, the present author has re-investigated the Li effects on the potential and extended the experiments to include also electrical impedance measurements.

The significance of the observed "Li-rhythms" is still obscure, but as it appears very regularly, one may perhaps assume, that it somehow is concerned with the fundamental ionic transport mechanisms of the skin and therefore worthy of the extensive report to be given in this paper. Below we will first describe the technique employed and then some typical results obtained with Li and alkali ions on the potential and impedance characteristics. Further experiments including measurements of ionic fluxes and effects of applied DC current are in progress.

Technique.

The *skin* was taken from the ventral side of *Rana temporaria* (or *R. esculenta*). Animals kept in a froggery over the winter months (at 16° C) or freshly captured were employed. The later gave usually higher potentials. The excised piece was immediately washed in about 20 ml Ringer solution (after GRAY (1941)), gassed with 2—3 % CO_2

in 97—98 % O_2, and kept there for about half an hour before the mounting (18°—26° C). The skin was then placed between the two halves of a Perspex "*dual chamber*", in principle somewhat similar to that described by TEORELL and WERSÄLL (1945).

The construction is illustrated in the diagrams (Figure 1 and 2). It contains chambers for *two* separate outside solutions, (1) and (2), and one common inside solution (usually Ringer) divided in two equal, communicating volumes, (3) and (4). Each of the four chamber sections contains about 2.5 ml. The two parts of the apparatus are screwed together by four long screws (*f*). This device incorporates two pairs of built-in miniature calomel electrodes (*a*—*e*) for potential measurements, accommodations for two pairs of resistance electrodes (*j*) and inlets (*h*) for gas mixture (or circulation pumps).

The apparatus has the following features:

a) Either of the two separate chambers (1) and (2), facing the anatomical outside of the skin, could be loaded with a test solution and measured for mV, resistance and ionic composition independently of the other. Usually the parts of the skin membrane exposed in the two adjacent holes behaved rather identically, whereby one chamber pair could serve as "control" to the other. b) With regard to experiments on the effects of electrical current flow (to be described in later communication), the "shock" or "stimulating" electrodes are placed in the separate outside chambers (1) and (2). When the shock potential is applied on these electrodes, the current will flow inwardly in one membrane section, for instance across (1)—(3), and outwardly in the other section (2)—(4). Thus this device simulates the situation when two stimulation electrodes are applied on the outside a nerve or a *Nitella* algae and one has the possibility of analysing the events under the "anode" and "cathode" separately.

The *calomel electrodes* (Figure 1) have the conventional filling of Hg/Hg_2Cl_2/saturated KCl. The calomel-KCl layer (*a*) on top of the mercury (*b*) is solidified with 1 per cent agar-agar. The saturated KCl solution filling the electrode space is made to the consistency of a non-flowing paste by addition of about 10 per cent of viscous methyl-carboxy-cellulose (the agar and mcc fillings are pressed into the electrode, with the top screw (*e*) removed, in the hot state by means of a surgical syringe with a coarse needle). This use of a high-viscous paste filling obviates difficulties with leakage of KCl, furthermore the "liquid junction" can be easily renewed by expelling a small plug of the KCl-paste through the small opening *g* of the small channel leading to the skin by turning the hollow Perspex screw (*d*). It can then be properly positioned to about 1 mm below the opening (*g*) by turning the screw a trifle backwardly. The screws *d* and *e* have to be greased with vaseline, whereby creeping of the KCl is also prevented. These junctions have remained stable for hours and the electrodes last without any appreciable assymmetry for months (when not in use the dual chamber is filled with half saturated KCl and the four calomel electrodes are kept short-circuited).

The *resistance electrodes* (*j*) were made from 0.5 mm silver or platinum

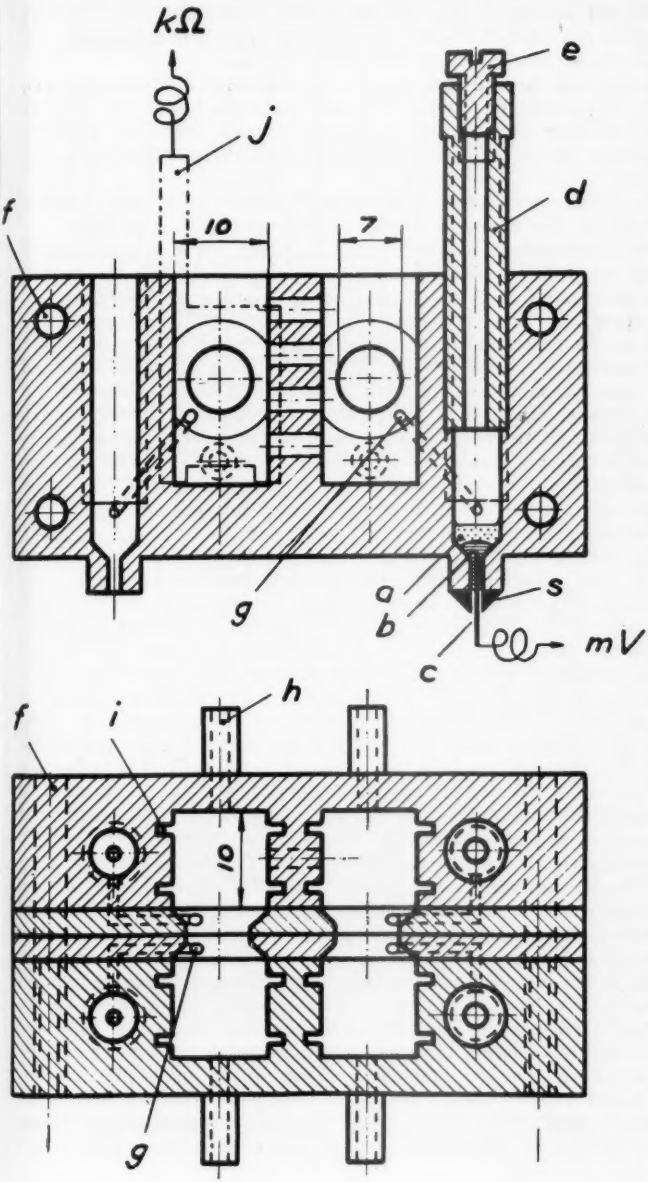

Fig. 1. *The "dual chamber" for frog skin (or membrane) experiments.* a) Calomel-KCl-agar layer, b) Mercury, c) Platinum wire, d) Hollow screw, for extrusion of KCl paste, e) Screw with hole for filling, f) Holes for clamping screws, g) Exit of channel from the calomel electrodes = "the liquid junction", h) Inlets for gas or liquid, j) Platinum electrodes + Pt black, i) Groves for Pt electrodes. — The material is Perspex. Thin rubber washers are used between the different sections for tightening. The scale is in millimeters.

sheeth and blackened with Pt-sponge electrolytically. They showed negligible polarisation above 10 c. p. s. and were here inserted in the groves closest to the skin, cf. Figures 1 and 2.

Gassing and *stirring* was secured by bubbling 2—3 per cent CO_2 in 97—98 per cent O_2 through the small pipes (*h*). In the experiments to be described in this paper the inside solution, chambers (3)—(4), was always Ringer solution according to GRAY. The temperature was 20—25° C.

The electrical measuring arrangement is pictured as a block diagram in Figure 2. The following details are of importance to mention:

The measurements are automatically timed by a selector switch (made up from multi-pole telephone relays operated by electromagnets actuated from a switching device joined to a "Brown 6-point, high speed, synchroprint recorder") and are recorded on the printing chart in the order: mV over (1)—(3), mV over (2)—(4), zero line (or mA), resistance (1)—(3), resistance (2)—(4). Each circuit is measured 3—4 times per minute and printed as points in different colours on the moving paper chart. In Figure 3 the "mV chamber (2)—(4)" curve is composed by the actual points recorded (as black dots) in order to illustrate that the density of the printed points is sufficient to form a continuous curve. When one circuit is being recorded, all the other circuits are open-circuited in order to avoid interferences.

The input resistance at the mV measurements is 1 MΩ, equal to the grid-earth resistance of the DC amplifier (General Radio Type 715-A). The sensitivity of the mV measurements is $\pm$ 0.5 mV, that of the impedance of the order $\pm$ 25 ohms. The leads from the resistance electrodes to the Wheatstone-bridge are effectively blocked by appropriate condensors in order to prevent flow from any DC sources or the calomel electrodes. The bridge-detector amplifier-rectifier chain feeds the unbalance of the bridge into the DC-amplifier + recorder. The unbalance is actuated by a proper choice of the impedance of the ratio arms of the bridge. The bridge is fed with 18 c. p. s. sinusoidal A. C. The alternating current is connected to the skin only during the ohm-recording (by aid of synchronised relays). The calibration is performed by means of reference resistances, which occasionally are switched in as substitute for the frog skin in the bridge.

Remarks on the impedance measurements: The choice of 18 c. p. s. A. C. for the resistance measurement is actually a compromise between different demands. As noticed already in previous papers, the "parallel" resistance, R_p, obtained from locus diagrams according to Gildemeister-Lullies-Cole (TEORELL 1949, cf. TEORELL and WERSÄLL 1945, TEORELL 1946), is the most sensitive indicator of impedance changes induced by salts or applied current flow. This quantity R_p, defined as the difference ($R_0 - R_\infty$), *i. e. DC resistance—high frequency resistance,* is not readily accessible for a continuous recording as required in these investigations. DC measurements alone would be easily feasible, but would involve "time-varying" or "delayed" rectifying effects (cf. TEORELL 1949, 1951, 1953). High frequency measurements would probably also be ambiguous as pure capacities in the object would

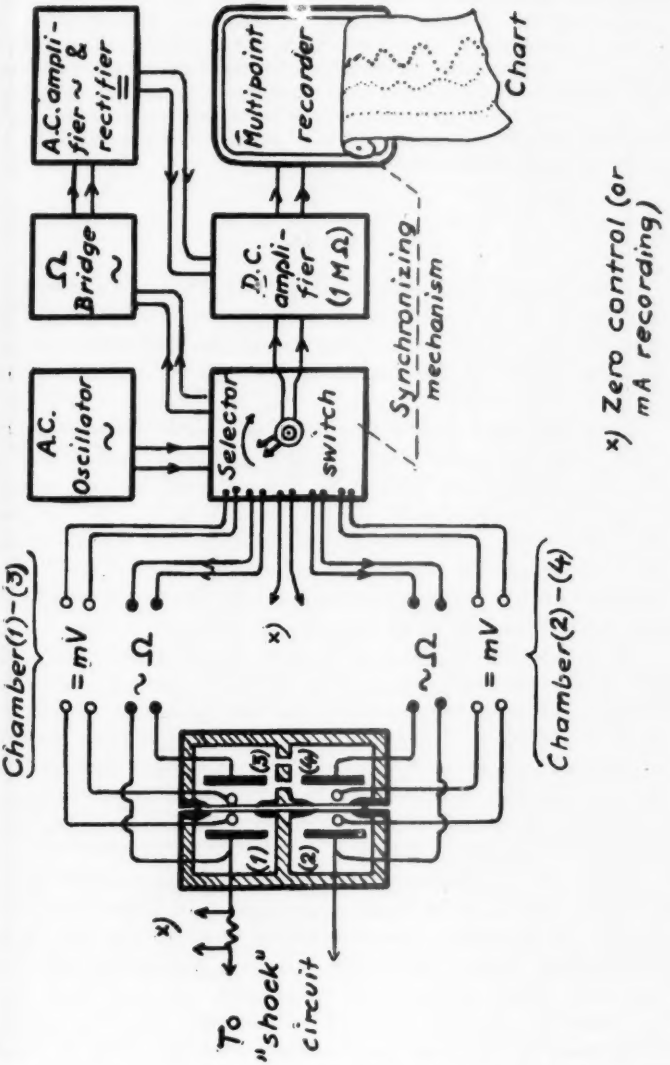

Fig. 2. *Block diagram of the recording arrangement.* For description see text.

short-circuit and swamp out possible changes in those "ohmic" ionic resistances, which are of primary interest to this work. The most suitable way out of these technical and theoretical difficulties seems to be the employment of a sinusoidal A. C. current of moderately *low* frequency. Appropriate considerations on the basis of the complete locus diagram from some frog skin experiments justify the choice of a frequency of 10—20 p/s, which yields an impedance vector approximately of the same magnitude as the "parallel" resistance ($R_0 - R_\infty$), cf. the experiments recorded in Figure 5.

Results.

1. *Experiments with lithium chloride:* In Figure 3 a record is reproduced, which shows the most typical features of a series of experiments made under varying conditions. The inside of the skin was bathed all the time in Gray-Ringer solution (chambers (3) + (4)).

When the same *Ringer* was also placed outside, the potential ("mV") and impedance ("kΩ") graphs remained approximately constant over a long period, as demonstrated by the control experiment (records "mV, (1)—(3)" and "kΩ, (1)—(3)" in the period 0—22 min.). This was also the case with the records across the skin section (2)—(4), which before the time "0 min." was treated with 100 mN NaCl in chamber (2) (which partly explains the higher initial levels of the (2)—(4) records).

When, however, the outside solution was changed to 100 mN *lithium chloride*, immediately there sets in a marked, *rhythmical variation* of both the *potential* and the *impedance*, as can be seen in Figure 3. When changing from Gray-Ringer, there is usually an immediate upward jump of the mean level of both mV and kΩ, then this level slowly declines with time. The superposed variations have a marked character of *a more or less pure sinusoidal, damped oscillation.*

As regards the *potential* behaviour the following comments can be made. The wave form is in this experiment rather pure sinusoidal, but in other cases it appeared often of the "full wave rectification" type. The amplitude always diminished with time and after about an hour, but often later, the wave train died away beyond the sensitivity of the measurements. The average magnitude of the potential "swings" is of the order of 5 mV in most experiments, less when the vital condition of the skin can be judged as less satisfactory. The periodicity of the waves

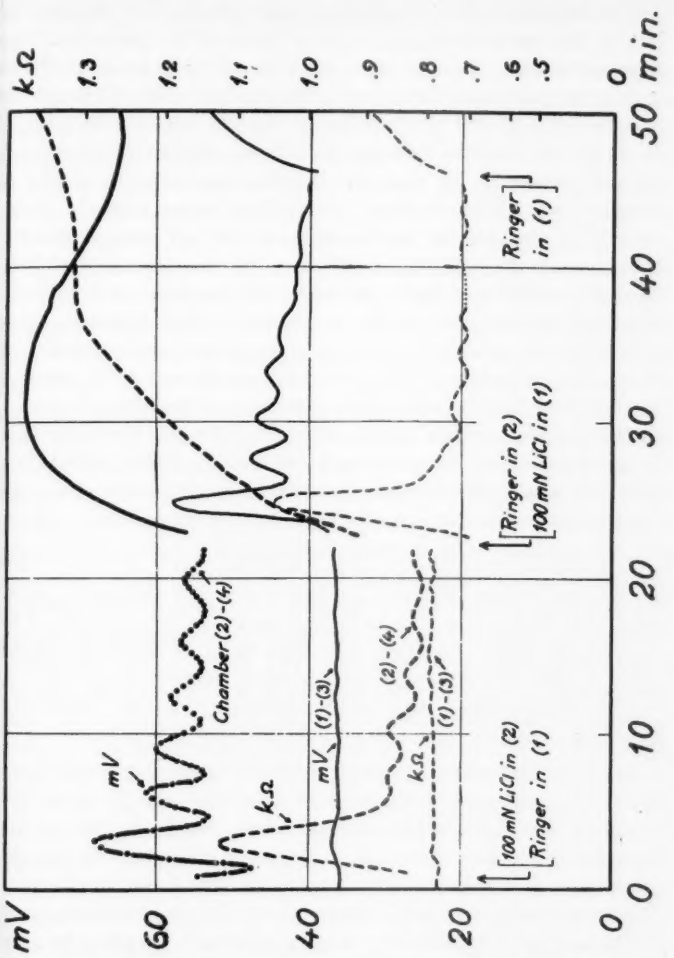

Fig. 3. *A typical experiment with LiCl and controls with Gray-Ringer solution on the outside of the frog skin.* Note the oscillations in potential (mV) (sign positive on the anatomical inside) and impedance (kΩ) in the Li experiments.

could in many experiments remain remarkably constant over periods up to an hour. In the record of Figure 3 the frequency was approximately 0.3 periods per *minute*, in other skins it could range between 0.1 to about 1 p/min.

The *impedance* (kΩ) oscillations were always less pronounced, but of the same sinusoidal type as those of the potential. They were quite easily observable in the example reproduced in Figure 3, but were in many other cases barely detectable, although the *average* level on the whole followed the mV curves. With regard to the phase relations between the mV and kΩ curves, the general feature seemed to be that the maxima and minima of the resistance (= 1/conductance) oscillations corresponded almost exactly to the minima and maxima of the mV waves. In other words, that *the potential oscillations are in phase with the conductance variations.* This conclusion is, however, not valid for the first "swing", as shown in Figure 3. The first mV wave, which here is unusually high, is accompanied by a *parallel* kΩ wave. The behaviour during the first minutes of LiCl exposure, as indicated by the experience also from other skins, points to rather complex events during this initial period. Probably there is a superposition of some extra electrical effects, which later fade out leaving the sinusoidal variations as the dominating ones. Further comments will be deferred to the discussion.

2. *Experiments with other alkali chlorides* (Na^+, K^+, Rb^+, Cs^+), H_4N^+ *and choline chloride:* In this paper we shall only briefly relate results from a series of experiments with exposure of the outside of the skin to other salts than those of Li, insofar as they bear on the discussion of the observed rhythmicity caused by the Li. The employed salts were all chlorides of sodium, potassium, rubidium, caesium and ammonium (usually 100 mN).

a) In the first place, the observations of the earlier investigators could be confirmed, that *only solutions rich in Na or Li could maintain an appreciable potential* across the frog skin (cf. the introduction). The potential depressing effect of K and the simultaneous impedance increase can be seen below in Figure 4. With frequently repeated washings of KCl (as well as of the other salts tested, including choline chloride) the potential could be pressed down to zero or even lower. This effect was quite reversible as Na or Li immediately restored a high potential (and lowered the impedance).

b) Secondly, it was found that *no other alkali ion, or ionic mixture tested so far, than those containing at least 10—20 mN Li could give rise to the oscillatory phenomena.*

Discussion.

The outstanding result of the experiments on the frog skins reported above is the appearance of a regular *rhythmicity* of the electrical *potential* difference and of the low frequency A. C. *impedance,* when the anatomical outside of the skin is exposed to solutions containing > 10 mN Li ions. An other feature is that these phenomena seemed to be specific effects induced only by Li. As regards the potential behaviour the findings of HASHIDA and TAKENAKA were thus confirmed, although the oscillation frequency in the Japanese experiments seemed to be much lower.

Rhythmicity and ionic transport. The pertinent question, which arises from these findings is naturally: what sort of structure, or system, in the skin operates periodically, or is conditioned to do so? In view of the experimental evidence produced in recent year chiefly by the Cambridge research group around HODGKIN, a Copenhagen group lead by USSING and also by LINDERHOLM in our laboratory, there seem to exist, in nerves and frog skins, mechanisms whereby metabolic processes can be coupled to transport of ions. The theories attempting to account for this so called "active transport" are indeed very vague so far and nothing definite is as yet known as to the chemical and physical nature of the proposed "ionic pumps" or "carrier" systems (cf. HODGKIN 1951, USSING 1952, CONWAY 1951—53). The leading theme in these modern works is that the formation of the electrical potential is, in the main, due to the actively transported sodium ions. As lithium ions are the only ones, which can yield potentials of the same order as Na ions, it would seem justified to conclude, that also Li ions are subject to some "active" transport mechanism. It should be pointed out that there exists as yet no direct evidence for this conclusion based on analytical influx and outflux studies on the Li ions. If one, however, takes an active Li transport for granted, it would appear tempting to suggest, that the rhythmical variations of the electric characteristics communicated in this paper would be due to a common mechanism on/in which *the Na and Li ions act in competition.* Simultaneous measurements of the Na and Li ionic fluxes would probably elucidate the validity of such a hypothesis (some attempts are under way in our laboratory). With the scarce evidence at hand at present, it is therefore certainly premature to advance any further hypothetical explanation of the rhythmical effects arising

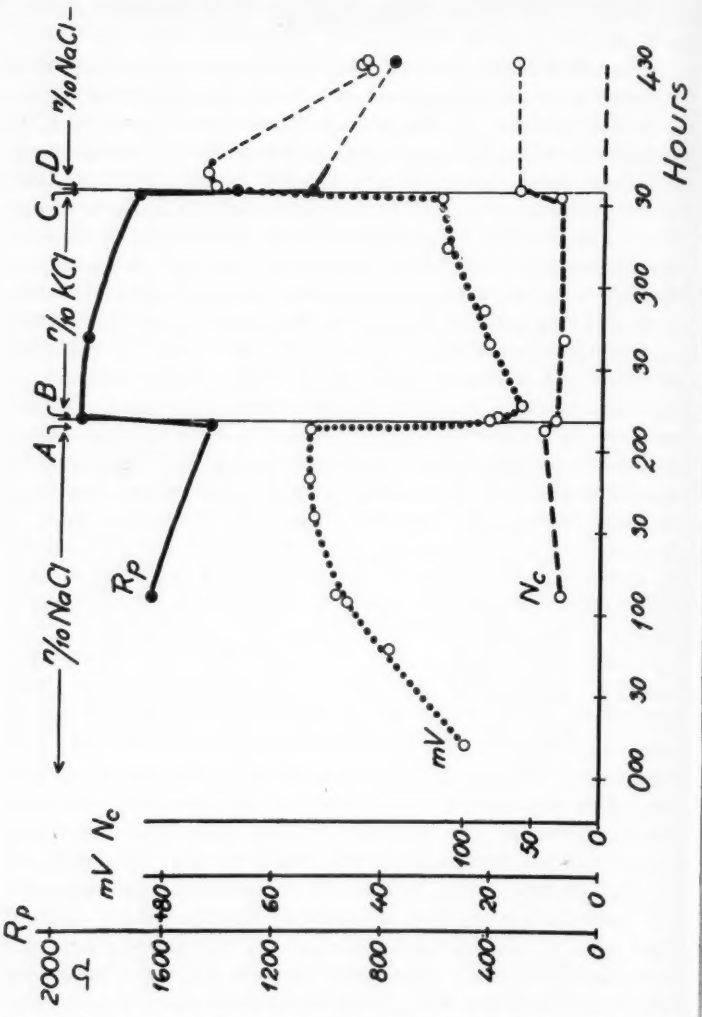

Fig. 4. *The effects of NaCl and KCl on the electrical behaviour of frog skin.* mV = the potential. R_p = the parallel resistance. N_c = the "characteristic" frequency (c. p. s.). The skin area in this experiment was 1 cm².

from the Li ions. Nevertheless, it might be valuable to pursue the discussion in somewhat different directions as follows below.

Interrelations between the potential and the impedance. Any future hypothesis on the source of the potential rhythmicity must account also for the concomitant impedance changes. Approximately the results reported above indicated that the periodical changes of the skin potential were accompanied by parallel changes in the conductance. Exceptions occur, however, as pointed out above, during the first period of the Li exposure. In a few skin experiments we have also noticed tendencies to "out of phase" rhythms of the potential and conductance waves. Furthermore, the amplitudes of the impedance waves can be quite different from skin to skin, although the mV waves can be similar in height. The reason for these confusing observations is obscure.

In connection with these comments on the impedance behaviour, it will be proper to recall that it has been observed earlier that frog skin potential and impedance (DC resistance) can vary simultaneously, but in opposite manner. From TEORELL (1946, Table I) we reproduce as a diagram, Figure 4, showing how the potential depression induced by KCl was accompanied by an elevation of the resistance (*i. e.* decrease of the conductance). The effect was reversible when again exposing the outside of the skin to NaCl. Other types of experiment, for instance those on the effect of aminophylline by LINDERHOLM (1952, Fig. 16), show, however, that the impedance (conductance) can change appreciably while the potential remains constant. Apparently, the interrelations between potential and impedance can be quite complex. We shall therefore at present refrain from any discussion of the possible biological factors involved and instead turn to the more physical question *i. e.* which impedance components are really measured with the use of low frequency sinus currents as used in the technique described in this paper?

Salt effects on the impedance components. The question raised in the previous paragraph is best elucidated by examining an A. C. "locus diagram" of frog skins, where a wide frequency range was employed. In Figure 5 are plotted loci diagrams corresponding to the Na-K exchange experiment represented in the previous Figure 4. The technique in these skin experiments was, in the main, the same as employed in the Li experiments of this paper, the impedance measurements were, however, made by means of the "square wave analysis" method according to TEORELL (1946).

On examining Figure 5 it is first observed, that both NaCl and KCl yield almost perfect semicircular loci-diagrams.

This allows us to interpret, at least formally, the impedance behaviour of the frog skin as due to an approximately *loss free* capacitive element shunted by an ohmic resistance R_p (of the order of 1,200—2,000 ohms). The high frequency resistance R_∞ of about 150 ohms is mainly due to the external resistance re-

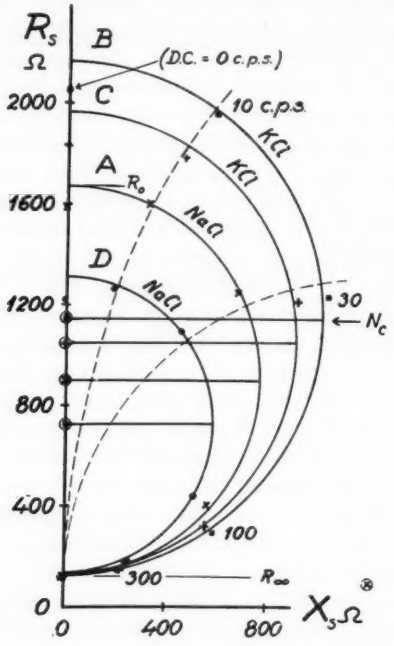

Fig. 5. *Impedance locus diagrams of a frog skin exposed to NaCl and KCl.* The same experiment as Figure 4. The markings A—D correspond to similar time markings of Fig. 4. The actual results from the "square wave analysis" are the dots and crosses. The applied square wave was 20 mV and of 70 c. p. s. R_s = equivalent series resistance. X_s = equivalent series reactance. R_0 = DC resistance (= 0 c. p. s.). R_∞ = high frequency resistance. N_c = the "characteristic" frequency. Skin area = 1 cm².

siding in the solutions between the electrodes and the skin. Secondly, one notices, irrespective of the treatment of the skin, that the impedance vector points for any particular frequency (for example 10 or 30 c. p. s. are located on another semicircular locus (the dashed curves in the figure). This property indicates that *the capacitive element always remains constant* (cf. the

observations on *Nitella* and *Loligo* by COLE & CURTIS (1938—39) and TEORELL's (1946) finding on frog skin that electrical stimulation did not markedly affect the capacitance).

From the "characteristic frequency", N_c, one can calculate the magnitude of the capacitance using COLE's formula $C_p = 1/2\ \pi N_c R_p$. At the time marked A in Figure 4 one obtains 4 to 5 $\mu F/cm^2$. This value is of the same order as observed on nerve axons or on *Nitella* by other authors. The nature of these remarkably high capacities is at present obscure. In this particular case of frog skin, it would be tempting to locate the capacity in a thin "basal membrane", which HASHIDA and his pupils and MEYER and BERNFELD discussed as a possible site of the potential generation (cf. also LINDERHOLM, USSING (1948)). Of a special interest in this connection are the recent micro-electrode experiments and electron-microscope studies by OTTOSSON, SJÖSTRAND, STENSTRÖM and SVAETICHIN. These authors present convincing, direct evidence for the existence of a "critical", thin membrane layer at the boundary between the epidermis and the corium. Although this "basement membrane" is almost submicroscopically thin, it remains doubtful, whether it has the proper physical properties to provide an almost loss free capacitance as found in our measurements. An other possibility is that one deals with an "apparent capacity" in the sense advocated by COLE (1947) and by the present author (1949, 1951, 1953).

From the discussion above it seems justified to conclude that the *low frequency impedance recorded in the experiments on the Li rhythmicity approximately represents the DC resistance, i. e.* an ohmic shunt resistance R_p, (cf. the impedance vectors of 10—30 c. p. s. are less than about 20 per cent shorter than the real R_p values located on the y-axis in Figure 5). The usual convention is to regard the R_p as a measure of the ionic permeability. We therefore provisionally state that *the rhythmical impedance changes observed with the Li solutions placed on the outside of the frog skin signify real ionic permeability variations*, which run parallel with the potential oscillations. It remains, however, to examine these permeability variations more in detail before the nature of the observed rhythmicity can be defined more satisfactorily. Experiments with tracers might be helpful.

Note on rhythmicity in artificial systems. Finally, it may be mentioned that the author recently has observed rhythmical potential and impedance variations in a purely artificial system, where a charged, porous membrane separating electrolyte solutions was subject to flowing electrical current. A complex interaction between electro-osmotic water streaming and ionic exchange resulted here in potential and conductance oscillations, usually of a sinusoidal, damped type. Under special, rather sensitive conditions, one could even observe practically un-

damped trains of potential and conductance waves lasting over long time periods. An essential phenomenon in these experiments was the occurrence of *a periodic water streaming* back and forward through the model membrane (unpublished experiments). Although these model observations may have no relevance to biological rhythmicity[1] as exposed in the Li experiments on frog skin related above, or to the well known oscillatory behaviour of nerve actions (cf. in this connection the communications by LUNDBERG (1951—52) on Rb and Li effects on nerves), they certainly hint to the necessity of examining in the future also the possible fluxes of water in these biological systems.[2]

Summary.

1. A technique is described permitting rapid automatic and continuous recording of electrical potential and A. C. impedance changes in isolated frog skin (or other membranes).

2. When the outside of an isolated frog skin was exposed to a > 10 mN lithium chloride solution *rhythmical* variations of the electrical potential as well as of the electrical conductance occurred. Usually the rhythm had the character of a slightly damped, sinusoidal oscillation lasting for hours. The amplitude of the potential waves was of the order 5 to 10 millivolts, the conductance variations amounted to about $\pm$ 10 per cent. The oscillation frequency was regular at about 0.3 periods per minute.

Earlier findings showing that only sodium and lithium salts could maintain a high skin potential were confirmed. Potassium, rubidium, caesium, ammonium and choline salts were "depolarizing" on the potential, simultaneously they depressed the skin conductance.

3. The observed A. C. impedance changes could probably be ascribed to variations in the resistive component, *i. e.* the ion permeability properties are subject to changes, while the capacitive components remain practically unaffected. The nature of the Li oscillations is discussed.

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[1] Various theoretical formulations of the physical conditions for the appearance of potential oscillations in biological systems have been attempted among others by MONNIER (1952), HODGKIN, HUXLEY and KATZ (1949), SHANES (1951) and KARREMAN and LANDAHL (1952—53).

[2] A recent work on the passage of D_2O across frog skin is that of KOEFOED-JOHNSEN and USSING (1953).

Literature.

Cole, K. S. and H. J. Curtis, J. Gen. Physiol. 1938. *22*. 37.
— Ibidem 1939. *22*. 649.
— Four Lectures on Biophysics. Instituto de Biofisica, Universidade do Brasil, Rio 1947.
Conway, E. J., Science 1951. *113*. 270.
— The Biochemistry of Gastric Acid Secretion, Charles C. Thomas, Publisher, Springfield, Ill. 1953.
— Internat. Rev. of Cytology, Vol. II, 1953, p. 419. Acad. Press, New York.
Galeotti, G., Z. phys. Chem. 1904. *49*. 542.
Gray, J. S. and J. L. Adkison, Amer. J. Physiol. 1941. *134*. 27.
Greven, K., Pflüg. Arch. ges. Physiol. 1941. *244*. 365.
Hashida, K., J. Biochem., Tokyo 1922. *1*. 21.
Hodgkin, A. L., Biol. Rev. 1951. *26*. 339.
— and B. Katz, J. Physiol. 1949. *108*. 37.
— A. F. Huxley and B. Katz, Arch. Sci. Physiol. 1949. *3*. 129.
Huf, E., Plüg. Arch. ges. Physiol. 1935. *235*. 655.
— Ibidem 1936a. *237*. 143.
— Ibidem 1936b. *238*. 97.
Huf, E., J. Parrish and G. Weatherford, Amer. J. Physiol. 1951. *164*. 137.
— and J. Parrish, Ibidem 1951. *164*. 428.
— and J. Wills, Ibidem 1951. *167*. 255.
— J. Gen. Physiol. 1953. *36*. 473.
Huxley, A. F. and R. Stämpfli, J. Physiol. 1951. *112*. 476.
Karreman, G. and H. D. Landahl, Cold Spring Harbor Symp. on Quant. Biol. 1952, Vol. XVII, p. 293.
— Bull. Mathematical Biophysics 1953. *15*. 83.
Koefoed-Johnsen, V. and H. H. Ussing, Acta Physiol. Scand. 1953. *28*. 60.
Krogh, A., Scand. Arch. Physiol. 1937. *71*. 60.
— Proc. Roy. Soc. B, 1946. *133*. 140.
Linderholm, H., Acta Physiol. Scand. 1952. *27*. suppl. 97.
Lundberg, A., Ibidem 1951. *22*. 365.
— Ibidem 1951. *23*. 263.
— Ibidem 1952. *26*. 156.
Meyer, K. H. and P. Bernfeld, J. Gen. Physiol. 1946. *29*. 353.
Monnier, A.-M., Cold Spring Harbor Symp. Quant. Biol. 1952. Vol. XVII. 69.
Motokawa, K., Jap. J. Med. Sci. III. Biophys. 1935. *3*. 69, 95, 117, 145, 177 and 203.
— Ibidem 1938. *5*. 67, 95, 111, 175, 197, 211.
Ottosson, D., F. Sjöstrand, S. Stenström and G. Svaetichin, Acta Physiol. Scand. 1953. *29*. suppl. 106, 611.
Overton, E., Pflüg. Arch. ges. Physiol. 1902. *92*. 346.
Shanes, A. M., Fed. Proc. 1951. *10*. No. 3, 611.

TAKENAKA, S., Jap. J. Med. Sci. III. Biophys. 1937. *4*. 143, 283.
TEORELL, T., Acta physiol. Scand. 1946. *12*. 235.
— Arch. Sci. Physiol. 1949. *3*. 205.
— Z. Elektrochem. 1951. *55*. 460.
— Progress of Biophysics 1953. Vol. III, p. 307.
— and R. WERSÄLL, Acta physiol. Scand. 1945. *10*. 243.
USSING, H. H., Nature 1947. *160*. 262.
— Cold Spring Harbor Symp. Quant. Biol. 1948. *XIII*. 193.
— Physiol. Rev. 1949. *29*. 127.
— Advanc. Enzymol. 1952. *13*. 21.

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On the Vibrational Sensitivity in Different Regions of the Body Surface.

By

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Received 15 January 1954.

Since the beginning of this century determination of vibratory thresholds in different regions of the skin have been carried out with the aid of tuning forks (e. h. RYDEL and SEIFFER 1903). In these investigations the threshold was expressed by the time during which the vibration — gradually diminishing in amplitude — was felt after the shaft of the tuning fork had been placed on the region of the skin to be examined. The first real determinations of the threshold amplitudes of vibration sense were made by KATZ and NOLDT in 1926. At a frequency of 50 Hz the threshold values in the finger tips varied between 0.3 and 0.9 μ. In 1935 SETZEPFAND determined the vibratory threshold amplitudes in finger tips at the frequencies 15—700 Hz. According to his investigation the optimum sensitivity is found between 200 and 250 Hz, where the thresholds are only 0.08 μ. LAIDLAW and HAMILTON (1937) determined the vibratory thresholds at 60 Hz in different cutaneous regions of sixty healthy persons. They found that the threshold amplitudes were very inconstant in different persons even when the same region was under investigation. In old and obese persons the thresholds were especially high. Lately BUGARD (1952) investigated the vibratory thresholds in ten subjects aged 25—40 years. Using a magneto-strictive vibrator he found that the lowest threshold values were obtained at 400 Hz being 0.11—0.13 μ in the fingers and palms, whereas in the elbow, sternum and precordial regions the threshold is 0.19 μ.

The experimental part of the present work was carried out in 1933—1934. Using a specially constructed and up-to-date electrodynamic vibrator a general investigation of vibratory thresholds was made; the range of the frequency spectrum was 25—1,280 Hz. Just when the results were to be published as an academic treatise the above-mentioned work of SETZEPFAND appeared, largely and justly detracting from the novelty value of the author's work. Both the methods and the results obtained by SETZEPFAND were almost identical with those of the author, who subsequently abandoned this field and went over into acoustics. Although during the eighteen years since SETZEPFAND's publication some additional information on the problem has appeared in the literature further knowledge is required on the vibrational sensibility in the different regions of the body and at different frequencies. From the author's file of records from old vibration experiments sufficient information on this problem can be found to justify a publication, especially since there is some disagreement between the values published by BUGARD and some of those given below.

Methods.

The sinusoidal alternating current for feeding the vibrator were obtained from a push-pull coupled generator furnished with very large coils for the lowest frequencies. The apparatus has been described in connection with the author's subsequent works (WILSKA 1935 and 1938). There was an instrument for measuring the current in the vibrator circuit, and the relation between this current and the vibration amplitude was carefully calibrated with a microscope.

The damping influence of the skin on the vibration amplitude was also taken into account. In order to transmit the vibration on the skin a cylindrical piece of wood, 10 mm in length and 1 sq cm in area was attached to the moving coil supporter. Due to the small size of the vibrating surfaces the apparatus was practically silent at frequencies below 200 Hz. When working at higher frequencies the ears of the subjects were stopped with wax.

During the determination of thresholds the wood cylinder of the vibrator was placed against the skin area to be examined, without applying excessive pressure. During the course of experiments it became clear that even considerable variations in this pressure had very little influence on the threshold values obtained. The frequencies used were 25, 45, 77, 125, 200, 270, 360, 450, 580, 750, 860, 1,020, 1,150 and 1,280 Hz. Above 1,280 Hz the amplitude obtained from the apparatus was too small to reach the threshold values that grow with the increasing frequency.

A healthy male subject, aged 22, was used for most of the experiments. Many of the results were compared with those obtained from two other subjects of the same age and sex. No appreciable differences were found, especially if the normal daily variations of thresholds by a factor of 5—10 are taken into account. Similar threshold fluctuations are also described by SETZEPFAND.

Results.

In the main series of experiments concerning the vibratory sensitivity of finger tips it was found that if the size of the vibrating area is reduced from 100 to 10 or to 1 sq mm the thresholds remain very much the same up to a frequency of 270 Hz. At higher frequencies the thresholds are 2—3 times higher when using the 1 sq mm area than when using the larger ones. The threshold amplitudes are the lowest in the frequency range 200—450 Hz, amounting to 0.1 μ on an average, whereas in single experiments values down to 0.02 μ have been obtained at the frequency 270 Hz. From this optimum the threshold amplitudes rise towards both ends of the frequency scale, first slowly and then faster. At the frequencies 77 and 860 Hz the 1 μ mark is reached and at about 25 Hz the threshold curve crosses the 10 μ line. As mentioned above, these values refer to the finger-tips only.

Results from the experiments on the vibrational sensitivity of the different regions of the body surface are summarized in Table 1. From the threshold amplitudes obtained at different frequencies a separate diagram was made for each region. By the use of this diagram it was easy to ascertain threshold values for the frequencies 50, 100, 200, 400 and 800 Hz and simultaneously to smoothe out many occasional irregularities found in the original curves at neighbouring frequencies. This done, and when the different regions investigated were placed in the order of their sensitivity, the originally very confusing list of data became easier to survey and comprehend. The regions were named according to SPALTHEHOLZ (1922). In all these experiments, the 100 sq mm vibrator was used. The stimulation was applied in the middle part of each anatomical region. From Table 1 we can see that the hand is the region most sensitive to vibration. Next comes the sole of the foot. This is not only interesting from a phylogenetic point of view but also because the vibrational sen-

Table 1.

Regio	Vibratory threshold amplitude in μ at				
	50 Hz	100 Hz	200 Hz	400 Hz	800 Hz
Volaris digitorum manus	2.0	0.6	0.07	0.05	0.3
Volaris manus	2.5	0.7	0.07	0.06	1.1
Dorsalis manus	4.1	1.8	0.11	0.16	5.5
Antebrachii ulnaris	4.0	1.2	0.28	0.15	5.4
Antebrachii dorsalis	4.3	1.6	0.42	0.32	7.2
Plantaris pedis	6.5	1.5	0.45	0.36	7.1
Antebrachii volaris	7.6	1.8	0.39	0.72	>10
Sternalis	7.6	3.8	0.28	0.6	5.8
Plantaris digitorum pedis....	8.8	3.7	0.77	0.74	>14
Malleolaris lateralis	18	11	1.8	0.6	13
Olecrani	25	7.2	1.3	0.9	11
Malleolaris medialis	12	6.2	1.4	1.1	14
Calcanea	4.2	0.9	1.8	14	—
Brachii posterior	21	12	1.1	5.6	>18
Brachii anterior.....	5.3	4.2	3.2	1.6	>16
Scapularis	11	4.5	1.4	11	—
Femoris anterior	12	6.4	1.8	16	—
Mammalis	7.2	4.0	1.7	3.8	>18
Oralis	19	8.0	2.2	6.1	—
Cruris anterior	41	14	2.5	8.2	—
Cruris posterior	12	5.4	2.8	12	—
Nuchae	10	5.6	3.1	3.1	6.9
Patellaris	95	31	5.6	3.3	>18
Lumbalis	13	8.1	4.2	13	>18
Frontalis	19	14	4.2	7.4	—
Nasalis	4.1	7.8	4.7	20	—
Parotideo-masseterica	5.1	7.4	6.3	29	—
Mentalis	14	8.6	5.6	17	—
Suralis	23	11	5.6	27	—
Laryngea	36	12	5.6	18	—
Epigastrica	35	10	5.9	9.6	—
Hypogastrica	56	11	4.5	31	—
Coxae	115	29	5.6	18	—
Glutaeae	40	27	14	>60	—

sitivity of the planta pedis may be very important for normal walking reflexes, especially for the more or less subconscious perception of the consistency of the soil surface. The sternal region of the chest wall is relatively sensitive, too. This may explain the perception of musical tones by some deaf-mutes as vibrations in their chest wall, as described by KATZ (1935). The region of the larynx, which vibrates at each phonation, is as insensitive as the abdomen. The distal parts of the extremities are more sensitive than the proximal ones. The lips are not especially sensitive to vibration, and the threshold values on the head are unexpectedly high as a whole. The least sensitive part of our body is the gluteal region.

Summary.

Vibratory thresholds in different parts of the body were determined with an electro-dynamic apparatus. The lowest threshold amplitudes were found within the frequency range 200—450 Hz. In the finger-tips these may be as small as 0.02 μ. Hands and the soles of the feet are most sensitive, whereas the vibratory threshold values are highest in the abdominal and the gluteal regions.

Literature.

Bugard, P. J., J. physiol. Path. gén. 1952. *44*. 230.
Katz, D., Z. Psychol. 1925. Erg. Bd. *11*. 1.
— and F. Noldt, Ibidem 1926. *99*. 104.
Laidlaw, R. W. and M. A. Hamilton, Bull. Neurol. Inst. N. Y. 1937. *6*. 494.
Reydel, A. and W. Seiffer, Arch. Psych. 1903. *37*. 488.
Setzepfand, W., Z. Biol. 1935. *96*. 236.
Spalteholz, W., Handatlas der Anatomie. Bd. 2. 1922.
Wilska, A., Skand. Arch. Physiol. 1935. *72*. 161.
— Untersuchungen über das Richtungshören. Helsinki 1938.

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The Hypothalamus, a Relay Station of the Sympathetic Vasodilator Tract.

By

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Topical electric stimulation in the anterior part of the hypothalamus was observed to elicit vasodilatation in the skeletal muscles of the cat and dog (ELIASSON, FOLKOW, LINDGREN and UVNÄS 1951, ELIASSON, LINDGREN and UVNÄS 1952). The vasodilatations were due to the activation of sympathetic cholinergic vasodilator nerves and could be blocked with atropine. Concomitant vasoconstrictions occurred in the skin and the intestines. In the cat, additional manifestations of sympathetic activity appeared, such as an increase in the heart rate and pupillary dilatation. The redistribution of the circulating blood from the cutaneous and splanchnic areas to the skeletal muscles, and the simultaneous discharges from other sympathetic outflows, suggested a reaction pattern characteristic of states of emergency.

Since the hypothalamus is a region considered to integrate activity within the sympathetic nervous system, ELIASSON et al. (1951) interpreted their observations to indicate that the sympathetic vasodilator outflow is also among the efferent nervous pathways under the integrative control of the hypothalamus. They suggested that one function of hypothalamic "vasodilator" area might be to produce vasodilatation in the skeletal muscles in cases of emergency, or in circumstances when a sudden increase

in muscle blood flow is necessary in order to create optimal conditions for muscular effort.

The existence of a corticohypothalamic vasodilator pathway indicates that the sympathetic vasodilator outflow is also under cortical control (ELIASSON et al. 1952).

If the hypothesis that the hypothalamus is able to initiate or influence sympathetic vasodilator activity is to hold good, vasodilator neurons must originate in the hypothalamus or be relayed from it. The aim of the present experiments was to throw light on this question.

Technique.

The observations were made on cats and dogs under chloralose (50—70 mg/kg) or dial (40—50 mg/kg) anaesthesia.

Topical electric stimulation in the hypothalamus was effected with a thin bipolar electrode, oriented by means of a stereotaxic instrument. Square wave impulses with a duration of 2 milliseconds, a frequency of 70 per second and an intensity of 1—4 volts were used for stimulation. The output resistance of the stimulator was 1,000 Ohms.

A direct method was used to measure the blood flow. From the cannulated vein, the blood was directed to a photoelectric drop counter operating an ordinate writer.

From the drop chamber, the blood ran back to the animal into a cannulated external jugular vein (for details of the method, see LINDGREN and UVNÄS 1954).

In experiments on cats, the blood flow from the muscles of a skinned hind leg was recorded in the femoral vein. In dogs, the blood flow was recorded in the popliteal vein. In the majority of experiments, measurements were made in both hind legs simultaneously.

The arterial blood pressure was recorded in a carotid artery with a mercury manometer. To prevent clotting, heparin was given intravenously, 0.2 ml/kg of a 5 per cent solution.

In three experiments on decorticate dogs, the spinal medulla was ligated at L_5. This operation denervates the hind legs but leaves their sympathetic innervation intact, since it emerges from segments above L_5.

In most experiments, bilateral vagotomy was performed in addition to occlusion of the common carotids, in order to eliminate the function of the pressor receptors.

Decortication. This operation was performed under sterile conditions on 6 dogs under nembutal anaesthesia (25 mg/kg I.V.). After incision of the skin, the skull was opened by means of a dentist's drill and a pair of bone forceps. The dura was sectioned on both sides and the dorsal surface of the brain exposed. A layer of brain tissue about 1 cm in thickness was removed by suction. After careful haemostasis, the dura was sutured with fine silk and the incision in the skin closed with

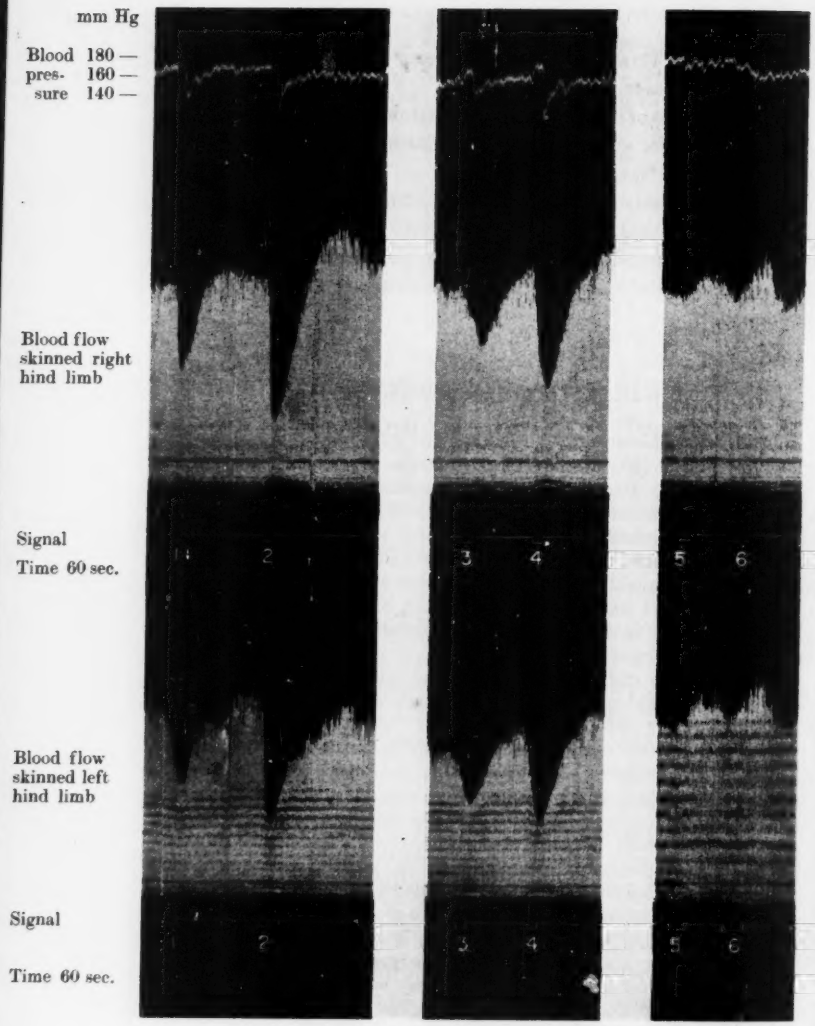

Fig. 1. Dog 6.7 kg. Vasodilator responses in the skinned hind legs to stimulation in the supraoptic area of the hypothalamus, 1.5 mm to the right of the midline. 1 and 2: stimulation before section of the right vasodilator tract (at B in Fig. 2).
3 and 4: » after » » » » » »
5 and 6: » » » atropine 0.1 mg/kg I.V.

Intensity: 1 and 3: 3 volts; 2, 4 and 5: 4 volts; 6: 5 volts.

Note: The vasodilator responses are still bilateral after unilateral section of the vasodilator tract. They disappear after atropinization.

steal wire. In order to secure postoperative nervous degeneration and full recovery, the animals were not experimented on until 3—6 weeks later.

Results.

Confirming earlier reports, stimulation in the supraoptic part of the hypothalamus was found to produce bilateral vasodilatation in the muscles of the hind legs in both cats and dogs. The responsive field was situated on either side of the midline a few mm above the optic chiasma. In fact, at the supraoptic level, vasodilator responses could be elicited even with the tip of the electrode placed in the midline. This observation indicates that connexions may exist between the "vasodilator" areas on each side of the midline. The possibility must nevertheless be borne in mind that a bilateral spreading of the stimulatory field might have occurred, owing to the position of the electrode in the midline.

From the supraoptic level, bilateral "vasodilator" tracts were found to run backwards through the hypothalamus. Passing the mesencephalon in a dorsolateral direction, they reached the collicular region where, at the level of the anterior colliculus, they were situated 2—3 mm below the dorsal surface.[1]

The vasodilator responses to stimulation of the hypothalamocollicular part of the vasodilator area were bilateral. The ipsilateral vasodilatations were, however, usually more pronounced than the contralateral responses. Slight changes in the position of the electrode were able to influence the quantitative relations between the vasodilator responses in the two hind legs, a fact which might reflect a spatial organization of the vasodilator supply to the two legs.

As mentioned previously, our results suggested the existence of anatomical connexions between the vasodilator areas in the supraoptic part of the hypothalamus. The following experiment shows that such connexions exist (Fig. 1). Bilateral vasodilator responses were elicited in the hind legs of a dog by stimulation in the right supraoptic vasodilator area (1 and 2 in Fig. 1). The tip of the electrode was situated 2 mm above the optic chiasma and 1.5 mm to the right of the midline. An attempt was then made to interrupt the vasodilator tract of the same side, behind the electrode, by pushing a metal strip from the dorsal cerebral

[1] More detailed anatomical data will be given in a later communication.

Fig. 2. Medial aspects of the dog's brain from the experiment illustrated in Fig. 1. The electrode (A) and the metal strip (B) used to sever the vasodilator tract were left and photographed in their original positions. The projection of the tip of the electrode on the medial surface is marked black.

surface down to the bottom of the skull. The strip was 12 mm wide, and if inserted vertically close to the midline with its cutting edge in the frontal plane, it was estimated to impinge on the vasodilator tract. The photograph in Fig. 2 shows the position of the electrode and of the metal strip. The strip had passed 3 mm behind the tip of the electrode. At the level of the vasodilator tracts, the lateral margin of the strip reached 10 mm from the midline; fortunately enough it extended beyond the midline and made an incision 1 mm deep in the diencephalon of the other side. Since at the level of the cut the vasodilator tract passes at most 4—5 mm from the midline, complete section of the right vasodilator tract was achieved.

As shown at 3 and 4 in Fig. 1, despite section of the right vasodilator tract, stimulation in the right supraoptic area still produced bilateral vasodilator responses.

Atropine, 0.1 mg/kg I.V., completely prevented further vasodilator responses (5 and 6 in Fig. 1).

The motor cortex was removed bilaterally in 6 dogs. The extent of decortication varied somewhat in the individual animals (Fig. 4), but in every case it included the area from which the sympa-

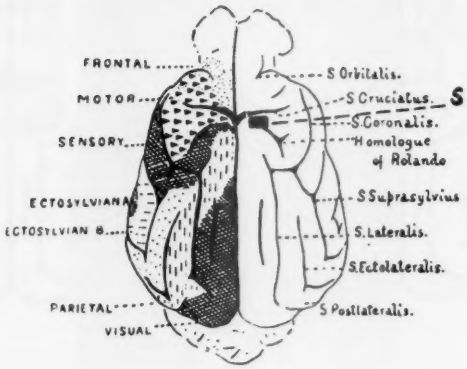

Fig. 3. Dorsal aspect of the brain of the dog. The area from which vasodilator responses could be evoked in the skeletal muscles is shaded (S) in the right hemisphere (from ELIASSON, LINDGREN and UVNÄS 1952).

thetic vasodilator neurons were observed to emanate (Fig. 3). The time elapsing between decortication and the experiment is shown in the following table.

Dog number	Time in days between decortication and acute experiment
1	20
2	22
3	27
4	41
5	42
6	42

The observations on the decorticate dogs showed that electric stimulation in the anterior part of the hypothalamus was able to produce vasodilatation in the skeletal muscles to the same extent as in animals with an intact cortex. The responsive region comprised the same hypothalamic area in the decorticate as in the normal dogs, and decortication was not found to cause any appreciable change in the vasodilator responses.

Fig. 5 shows the results in one of the decorticate dogs. Stimulation in the anterior part of the hypothalamus (1 and 2 in Fig. 5) caused vasodilatation in the muscles of a motor-denervated hind limb with its sympathetic outflow intact.

As described in previous articles, vasodilator effects due to sympathetic vasodilator impulses are blocked by atropine. In

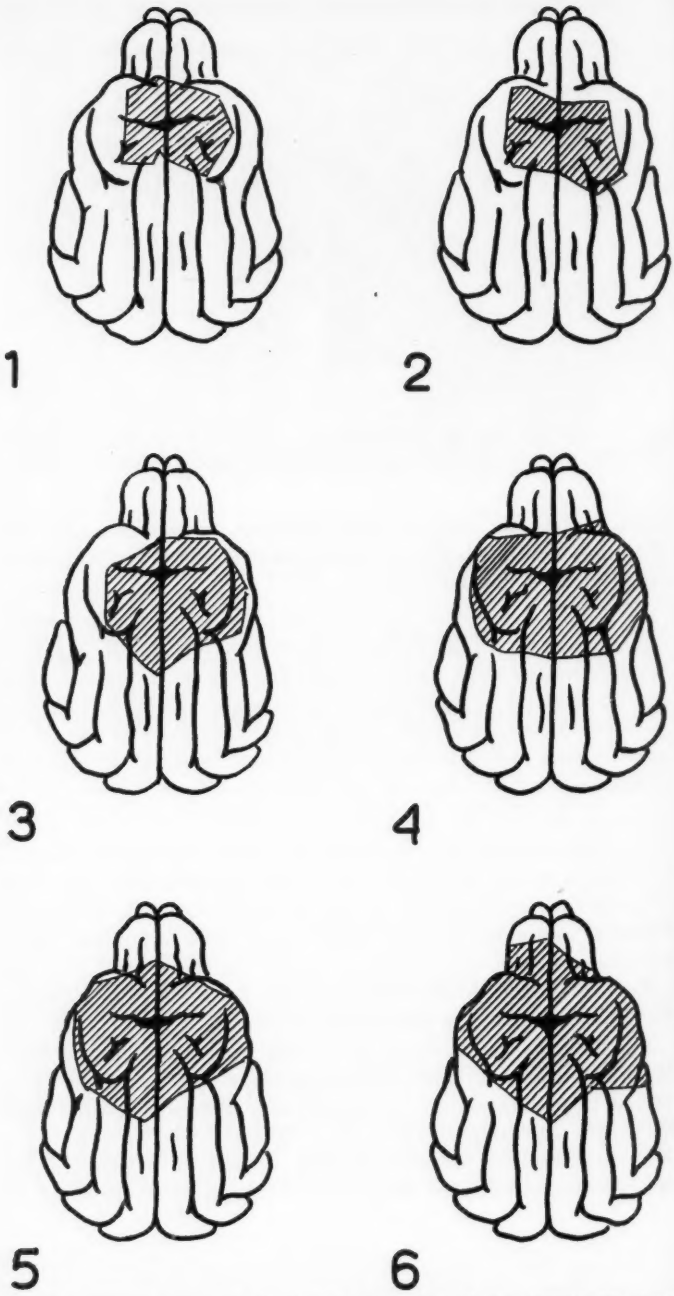

Fig. 4. Schematic drawings showing the extension of the decorticated areas (shaded).

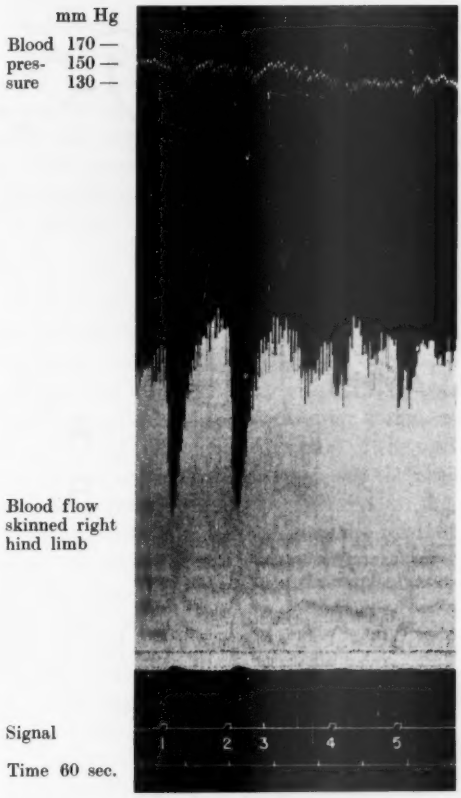

Fig. 5. Dog 6.3 kg. Vasodilator responses in the skinned right hind leg of a decorticate dog to electric stimulation in the anterior hypothalamus. Spinal medulla ligated at L_5.

1 and 2: before atropine.
4 and 5: after atropine, 0.1 mg/kg I.V. (at 3).

Intensity: 1.25 volts.

Note that atropine blocks the vasodilator responses to stimulation and converts the earlier depressor responses to pressor responses.

this experiment, 0.1 mg/kg I.V. of atropine was given to the animal (at 3 in Fig. 5); it is seen that an immediate change occurred in the stimulatory responses. Before the administration of atropine, hypothalamic stimulation produced a marked increase in muscle blood flow, and a moderate decrease in blood pressure.

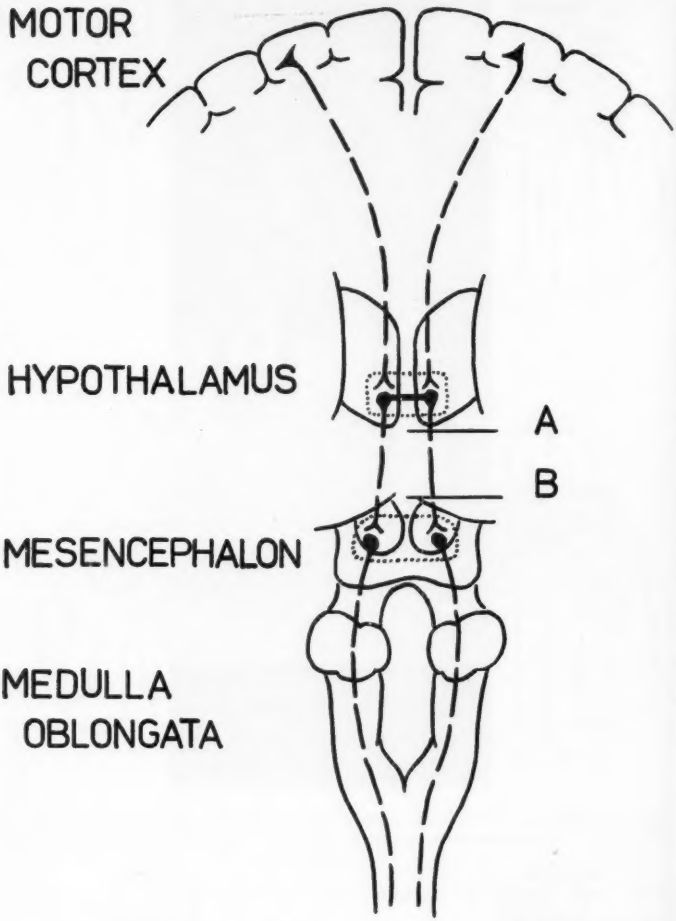

Fig. 6. Schematic drawing showing the intracerebral course of the sympathetic vasodilator tract, from its origin in the motor cortex via the hypothalamus, mesencephalon and medulla oblongata to the spinal medulla.

As discussed in earlier articles, the fall in blood pressure depends on the fact that the vasoconstrictions in the cutaneous and splanchnic areas do not suffice to compensate for the extensive vasodilatation in the muscles. After atropine was given,

hypothalamic stimulation resulted in only a slight increase in the muscle blood flow, and the depressor responses were replaced by pressor responses. As stressed in crosscirculation experiments (ELIASSON et al. 1952), the slight increase in blood flow in the atropinized animal is not produced by sympathetic vasodilator impulses, but is secondary to the pressor responses caused by the unmasked vasoconstrictor activity in the skin and the intestines.

Comments.

An account has been given in earlier papers of the cortico-hypothalamic and the medullo-spinal courses of the sympathetic vasodilator outflow (ELIASSON et al. 1952, LINDGREN and UVNÄS 1953). The results in the present report complement our knowledge with the hypothalamo-collicular part of the outflow.

The present experiments have shown the extension of the vasodilator tract from the supraoptic area in the hypothalamus to the collicular region. Stimulation in the supraoptic area produced bilateral vasodilator responses in the muscles of the hind limbs, as did stimulation of the intact vasodilator tract along its hypothalamo-collicular course. The bilateral responses to stimulation in the *supraoptic area* persisted after interruption of the tract immediately behind this area (A in Fig. 6, B in Fig. 2). On the other hand, LINDGREN (1954) observed in cats that section of the vasodilator tract immediately *anterior to the anterior colliculus* (B in Fig. 6) abolished the vasodilator responses to stimulation in the posterior part of the diencephalon. Subsequent stimulation at the same level *on the contralateral side* still caused bilateral vasodilator responses. LINDGREN and UVNÄS (1953) have earlier reported that stimulation of the vasodilator tract along its *medullary course* induced ipsilateral vasodilatation only.

Taken collectively, the observations described in the aforegoing justify the following conclusions. In the supraoptic part of the hypothalamus in the cat, there is anatomical connexion between the vasodilator areas on either side of the midline. From the supraoptic area, vasodilator neurons pass caudally, to cross or form synapses at some point between the anterior margin of the collicular area and the medulla oblongata.

ELIASSON et al. (1952) found that sympathetic vasodilator neurons originated in an area situated in the motor cortex, be-

tween the cruciate sulcus and a sulcus considered to be a homologue of the fissure of Rolando. From the cortex, a vasodilator tract could be traced down to the hypothalamus. Provided that the sympathetic vasodilator outflow has no other cortical representation, the fact that responsive vasodilator neurons persisted in our decorticate dogs indicates that sympathetic vasodilator neurons originate in the supraoptic area of the hypothalamus.

ELIASSON et al. (1951, 1952) proposed that the activation of the sympathetic vasodilator outflow might be controlled and integrated at the hypothalamic level. As was stressed in the beginning of the present paper, if this hypothesis is to hold good, vasodilator neurons must originate in the hypothalamus or be relayed from it. The present observations strongly indicate that they do.

Summary.

The hypothalamo-collicular extension of the sympathetic vasodilator tract is described. The observations indicate that the vasodilator neurons have synapses in the hypothalamus, and at some point between the anterior margin of the collicular area and the medulla oblongata.

References.

ELIASSON, S., B. FOLKOW, P. LINDGREN and B. UVNÄS, Acta Physiol. Scand. 1951. *23*. 333.
— P. LINDGREN and B. UVNÄS, Ibidem 1952. *27*. 18.
LINDGREN, P. and B. UVNÄS, Ibidem 1953. *29*. 137.
— — Circulation Research 1953. *1*. 479.
— — Acta Physiol. Scand. 1954. To be published.
LINDGREN, T., Personal communication.

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